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A Seasonal and Ontogenetic Examination of the Reproductive Biology of Pseudoplusia Includens (Walker).

Linda Jean Mason
Louisiana State University and Agricultural & Mechanical College

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Mason, Linda Jean, Ph.D.

The Louisiana State University and Agricultural and Mechanical Col., 1987

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A SEASONAL AND ONTOGENETIC EXAMINATION OF THE REPRODUCTIVE BIOLOGY OF *PSEUDOPLUSIA INCLUDENS* (WALKER)

A Dissertation

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy

in

Department of Entomology

by
Linda Jean Mason
B.S., University of Florida, 1979
M.S., Auburn University, 1983
May 1987

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This would not be complete without mentioning the two 'hairy' family members that were always waiting with a kiss and a wagging tail. To Chelsi and Buddy - big dog bones and a steak await both of you when I get a REAL job!

This labor of love I call my dissertation is dedicated to a very special women in my life - my grandmother - Phyllis Rose Elkins (1902-1986). Although you did not live to see me complete this project, your strength has seen me through the last few months and I know that I will always draw from it and the encouragement you gave. Thank you for being you.

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ABSTRACT

Laboratory observations on the mating behavior of soybean loopers, Pseudoplusia includens (Walker), indicated a calling pattern that was highly variable both within individual females over time and among individuals. However, some trends were apparent. The onset of calling showed temporal advancement over the first three nights of calling after adult emergence; the duration of calling showed a temporal advancement during the first six nights, while the termination of calling receded over the same period then reversed its trend. The majority of females initiated calling three nights following emergence while maximum percent calling was observed around five hours after the onset of scotophase. Spermatophore counts were highly correlated to the number of observed matings. Pairing relationships were less conclusive, although almost half of the multiple mating pairs exhibited mate fidelity.

Influence of tethered flight and carbohydrate deprivation on soybean looper whole body lipid, oviposition rate and survivorship was examined using a factorial experimental design. Food had a greater effect than flight on all three variables, although flight did decrease fecundity. Individuals that were subjected to only one stress, either two hours of daily flight or water as their only food source, were significantly different from both the no-stress group (nonflown and fed) and those that were both starved and flown. The general trend apparent for all parameters was nonflown-fed>flown-fed =nonflown-starved>flown-starved, except for stored lipids which

showed no statistical difference between flown-starved and nonflown-starved moths. Average longevity ranged from nine days for nonflown-fed moths to six days for flown-starved moths. These data demonstrated that the availability of a nectar source may be an important factor in the success of long distance soybean looper dispersal and subsequent reproduction.

Comparison of daily and seasonal values of whole body lipid, proportionate allocation of egg types, and age at first mating were examined in adult soybean loopers. Laboratory females possessed more whole body lipids than laboratory males. Laboratory individuals had a greater percentage of whole body lipids and number of chorionated eggs when compared to field-collected individuals. Late season females delayed chorionated egg production and had higher non-egg lipids than summer females. Both sexes contained a greater percentage of whole body lipids late in the fall when compared to early season moths. There was no shift in time of mating or number of matings between seasons. Thus, soybean loopers appear to exhibit a number of the characteristics associated with Johnson's oogenesis-flight syndrome proposed for migratory insects.

GENERAL INTRODUCTION

The soybean looper (Pseudoplusia includens (Walker))is a member of the subfamily Plusiinae and the tribe Plusiini (Hodges 1983). Its synonyms include: Plusia includens (Walker), Plusia dyaus (Grote), Plusia hemifera (Walker), Autographa culta (Litner), Autographa rogationis (Guenee), Autographa oo (Cramer), and Pseudoplusia oo (Cramer) (Eichlin and Cunningham 1978). The range of the soybean looper extends north from the West Indies and South America to Quebec, and west from Florida to California.

This insect can be a major economic pest of soybean, sweet potatoes and peanuts in the Southeastern United States (Hensley et al. 1964, Canerday and Arant 1967). It also has been found on cotton, tomatoes, crucifers, peas, tobacco, alfalfa, and collards (Crumb 1956, Mitchell 1967). Eichlin and Cunningham (1978) published an extensive list of host plants.

The adults are medium size moths (wingspan approx. 2.5cm) and are brown-grey in color. There is no obvious external characteristic to distinguish males from females although males can be determined by extrusion of genitalia.

The biology of the soybean looper was studied by Canerday and Arant (1967) and Mitchell (1967). Both examined the developmental rates for various life stages. Eggs are small and creamy white, and darken as the larve develop within. The eggs are deposited singly on the underside of leaves and hatch in approximately three days at 27^{o}C (Canerday and Arant 1967). The pale green larvae that emerge develop through six instars. Each of the first five instars is

completed in 2-3 days and the sixth in about six days. Mean total duration for the larval stage is between 13.4 days and 19.6 days at 27°C (Canerday and Arant 1967, Mitchell 1967). Mitchell (1967) found that development time for the larvae is faster when fed soybeans than if fed cotton. Shorey et al. (1962) reported that a late third instar male cabbage looper (Trichoplusia ni (Hubner)) larva can be distinquished from the female larva by the presence of two yellow testes located dorsolaterally in the fifth segment. This is also true for the soybean looper.

The larvae spend approximately 1.6 days spinning cocoons and as prepupae (Mitchell 1967). Pupation usually occurs on the underside of leaves and lasts approximately seven days. Pupae are variable in color, ranging from green to brown just prior to ecolsion. After ecolsion, adults spend 3-4 days in a preoviposition period. Adults are able to first mate during this period. It appears that the female is most attractive to males from midnight to 4 AM (Mitchell 1973). Canerday and Arant (1967) noted erratic egg production by soybean looper moths and suggested that failure in mating may be a causative factor. Virgin females usually lay fewer eggs than fertilized females and oviposition usually continues until death. Mason and Mack (1984) found that temperature had a significant inverse linear relationship with longevity and a significant curvilinear relationship with fecundity and daily egg production. Adult longevity at 27°C averages 12-14 days (Canerday and Arant 1967, Mitchell 1967).

Recently, Alford and Hammond (1982) examined soybean looper pheromone release as a function of temperature. For every ^{o}C decrease in temperature, sex pheromone release was shifted forward ca. 15 minutes during the first scotophase. Normal rhythms returned by the third scotophase. Peak calling occurred during the fifth hour of scotophase on the first day of calling and during the sixth hour on day three.

The effect of adult nutrition on oviposition, mating frequency, and longevity was examined by Jensen et al. (1974). They concluded that when cotton is a component of the soybean agricultural ecosystem, the nectar it furnishes to adult soybean loopers is a major reason for soybean looper outbreaks. Collins and Johnson (1985) examined the effects of weed nectar on fecundity in fall populations of soybean loopers. They found that a nectar source via weed nectaries resulted in an increased number of ovarian eggs and was thus important in reproductive success during the fall.

An annual migrant into Louisiana in late spring, the soybean looper overwinters in the United States in south Florida and south Texas (Mitchell et al. 1975, Newsom et al. 1980). It is unknown if overwintering populations contain both moths that have migrated from northern populations and year round southern residents or if these wintering populations are exclusively residents which expand their range each year. The former would fit Walker's (1980) return flight model, while the latter would fit in the diffusion and freeze-back model. Fall population studies suggest that there is a southerly flow, and adults have been trapped up to 100 miles out in the Gulf of Mexico (Lingren et al. 1979, Sparks et al. 1986). If a southerly migration occurs in the fall as soybean senesce, then

certain morphological and physiological changes associated with migration could occur. These changes may be part of the oogenesis-flight syndrome (Johnson 1969).

Morphological changes can include alary polymorphisms (Johnson 1963). An extreme case of alary change occurs in aphids, in which uncrowded

nonmigratory individuals are apterous while alates are produced under more crowded conditions (Dixon 1973). A similar case is found in some corixids and many other insects where polymorphism occurs in flight musculature without changes in the wings themselves. Flightless forms have thin white muscle fibrils compared to the large yellow fibrils found in flying forms (Young 1965). Gregarious locust have longer wings than less migratory solitary counterparts. Crowded pre-migrant Pieris brassicae (L.) and Plusia gamma (L.) have a lower wing loading ratio (i.e. large wing area relative to body weight) (Angelo and Slansky 1984).

Physiological changes include increased levels of flight fuels, i.e. stored lipid, and delays in egg production and mating (Johnson 1963, Kaster and Showers 1982, Solbreck 1972). Autumn blacklight trap captures of female black cutworms, Agrotis ipsilon (Hufnagel), in Iowa produced mostly unmated females, and sex pheromone traps had low captures of males when compared to flights at other times of the year. It is thought that the change in mating status during the autumn flight is an onset of reproductive diapause in preparation for a southward migration. Solbreck (1972) found that when Lygaeus equestris (L.) started autumn migration, fat body size was maximized, feeding had ceased, and sexual functions were delayed.

In general, premigrant males have no sperm in the seminal vessicles and also show increased fat storage (Johnson 1969).

Lepidoptera, Hemiptera, Coleoptera and Orthoptera usually use stored fat as the primary flight fuel during long migratory flights often breaking it down by enzymes in the flight muscles (Weis-Fogh 1952a, George and Bhakthan 1960). Fat, unlike carbohydrates, lasts longer during continuous flight, is stored without water, and produces eight times more energy for equal weights of glycogen (Weis-Fogh 1952a). During long migrations, glycogen or blood sugar may be used initially since it can be mobilized quickly. After the initial supply of carbohydrate is depleted, fat from the fat body and elsewhere is utilized. The fat may be rapidly stored after ecolsion as is the case for the monarch butterfly. Beall (1948) estimated that newly eclosed monarchs had fat reserves that were 30% of the lean weight whereas young migrants could have fat reserves of almost 125% of the lean weight. The fat reserves were depleted during flight to as low as 2% of the lean weight in adults caught in New Orleans after a long migration. The army cutworm, Chorizagrotis auxiliaris (Grote), also emerges with a low level of fat (5-15%) (Koerwitz and Pruess 1964) but accumulates fat prior to aestivation (Pruess 1967). Bogong moths (Agrotis infusa (Boisd.))start the aestivation period with high levels of fat (ca. 65% of lean weight) (Common 1954). Although fat levels appear to increase during the aestivation, this increase is probably a result of population changes at aestivation sites (Common 1954).

For some insects, migration can depress an individual's reproductive potential by increasing the risk of mortality or by

diverting energy reserves away from oogenesis (Roff 1977). This is particularly true for insects that use the same energy reserve for flight and reproduction. Unless these fat and carbohydrate reserves can be regenerated before oogensis starts or continues, fecundity can be affected. This reduction in fecundity was found to occur in Drosophila subobscura Collin (Inglesfield and Begon 1983). Weis-Fogh (1952b) found that in the desert locust, Schistocerca gregaria Forskal, overnight feeding did not always replace spent fat in flying females because the ovaries competed for the stored fat. It is unknown if soybean loopers energy reserves are affected by long term flight. However, soybean looper males will fly faster, but not longer, when they are young while female speed, duration, and distance decrease with age (Sharp et al. 1976).

The research in this dissertation was conducted to examine the reproductive biology of adult soybean loopers in an attempt to identify and characterize the oogenesis-flight syndrome. The need for increased levels of ecological, physiological, and behavioral studies of mobile noctuids was stressed recently in a review by Barfield and O'Neil (1984). It was hoped that this dissertation would add to the needed body of knowledge that is crucial in understanding the role of migration in the implementation of an integrated pest management program of pest control.

This dissertation is composed of four chapters. The first two chapters, examine, through a series of three experiments, calling and mating behaviors of soybean looper. The first experiment (Chapter 1) examines the qualitative aspects of soybean looper calling behavior, while the second experiment (Chapter 1) is a

quantitative examination of the influence of age on female calling behavior. The third experiment (Chapter 2) is an examination of mating behavior.

Chapter three examines the influence of tethered flight and food quality on soybean looper reproduction, survivorship, and whole body lipids. This was accomplished by a factorial experimental design with individuals either being flown or not flown, and given access to either water (starved) or a 15% sugar solution (fed). Because of the energetic demands of flight, it was postulated that the trend in lipid content and reproduction changes would be nonflown-fed > nonflown-starved ≈ flown-fed > flown-starved.

The final chapter is composed of a series of experiments examining daily and seasonal changes in stored lipids, proportionate allocation of egg types, and mating status. It was the aim of these experiments to test the existence of an oogenesis-flight syndrome in adult soybean loopers and then, if it existed, characterize it.

LITERATURE CITED

Alford, A.R. and A.M. Hammond, Jr. 1982. Temperature modification of female sex pheromone release in Trichoplusia ni (Hubner) and Pseudoplusia includens (Walker)(Lepidoptera: Noctuidae). Environ. Entomol. 11: 889-892.

Angelo, M.J. and F. Slansky, Jr. 1984. Body building by insects: Trade-offs in resource allocation with particular reference to migratory species. Fla. Entomol. 67: 22-41.

Barfield, C.S. and R.J. O'Neil. 1984. Is an ecological understanding a prerequisite for pest management? Fla. Entomol. 67: 42-49.

Beall, G. 1948. The fat content of a butterfly, Danaus plexippus Linn., as affected by migration. Ecology. 29: 80-94.

Canerday, T.D. and F.S. Arant. 1967. Biology of Pseudoplusia includens and notes on biology of Trichoplusia ni, Rachiplusia ou, and Autographa biloba. J. Econ. Entomol. 60: 870-871.

Collins, F.L. and Johnson, S.J. 1985. Reproductive response of caged adult velvetbean caterpillar and soybean looper to the presence of weeds. Agr. Ecosy. Environ. 14: 139-149.

Common, I.F.B. 1954. A study of the ecology of the adult Bogong Moth, Agrotis infusa (Boisd.) (Lepidoptera: Noctuidae), with special reference to its behaviour during migration and aestivation. Aust. J. Zool. 2: 223-263.

Crumb, S.E. 1956. The larvae of the Phalaenidae. USDA Tech. Bull. 1135. 366p.

Dixon, A.F.G. 1973. Biology of aphids. Edward Arnold (Publs.) London. 58p.

Eichlin, T.D. and H.B. Cunningham. 1978. The Plusiinae (Lepiodptera: Noctuidae) of America north of Mexico, emphasizing genitalic and larval morphology. USDA Tech. Bull. 1567. 122p.

George, J.C. and N.M.G. Bhakthan. 1960. A study on the fiber diameter and certain enzyme concentrations in the flight muscle of some butterflies. J. Exp. Biol. 37: 308-315

Hensley, S.D., L.D. Newsom and J. Chapin. 1964. Observation on the looper complex of the noctuid subfamily Plusiinae. J. Econ. Entomol. 57: 1006-1007.

Hodges, R.W. 1983. Checklist of the lepidoptera of America north of Mexico including Greenland. E.W. Classey limited and the Wedge Entomol. Res. Foundation. London. 284pp.

Inglesfield, C. and M. Begon. 1983. The ontogeny and cost of migration in Drosophilia subobscura Collin. Biol. J. Linn. Soc. 19: 9-15.

Jensen, R.L., L.D. Newsom and J. Gibbens. 1974. The soybean looper: effects of adult nutrition on oviposition, mating frequency, and longevity. J. Econ. Entomol. 67: 467-470.

Johnson, C.G. 1963. Physiological factors in insect migration by flight. Nature. 198: 423-427.

Johnson, C.G. 1969. Migration and dispersal of insects by flight. Methuen and Co., Ltd. London 763 pp.

Kaster, L. Von and W.B. Showers. 1982. Evidence of spring immigration and autumn reproductive diapause of the adult black cutworm in Iowa. Environ. Entomol. 11: 306-312.

Koerwitz, F.L. and K.P. Pruess. 1964. Migratory potential of the army cutworm. J. Kansas Entomol. Soc. 37: 234-239.

Lingren, P.D. T.J. Henneberry and A.N. Sparks. 1979. Current knowledge and research on movement of the cabbage looper and related looper species. In: Movement of Highly Mobile Insects: Concepts and methodology in research. R.L. Rabb and G.G. Kennedy (Eds.). pp. 394-405.

Mason, L.J. and T.P. Mack. 1984. Influence of temperature on oviposition and adult female longevity for the soybean looper Pseudoplusia includens (Walker) (Lepidoptera: Noctuidae). Environ. Entomol. 13: 379-383.

Mitchell, E.R. 1967. Life history of Pseudoplusia includens (Walker) (Lepidoptera: Noctuidae). J. Georgia Entomol. 2: 53-57.

Mitchell, E.R. 1973. Nocturnal activity of adults of three species of loopers, based on collections in pheromone traps. Environ. Entomol. 2: 1078-1080.

Mitchell, E.R., R.B. Chalfant, L.G. Greene and S.C. Creighton. 1975. Soybean looper: populations in Florida, Georgia, and South Carolina, as determined with pheromone-baited BL traps. J. Econ. Entomol. 68: 747-750.

Newsom, L.D., M. Kogan, F.D. Miner, R.L. Rabb, S.G. Turnipseed, and W.H. Whitcomb. 1980. General accomplishments toward better pest control in soybeans. In: New technology of pest control. pp. 51-98. C.B. Huffaker (Ed.) John Wiley and Sons, New York, 500 pp.

Pruess, K.P. 1967. Migration of the army cutworm, Chorizagrotis auxiliaris (Lepidoptera: Noctuidae). I. Evidence for a migration. Ann. Entomol. Soc. Am. 60: 910-920.

Roff, D. 1977. Dispersal in dipterans: Its cost and consequences. J. Ani. Ecol. 46: 443-456.

Sharp, J.L., J.R. McLaughlin and D.R. Bennett. 1976. Flight mill studies with the soybean looper (Lepidoptera: Noctuidae). J. Georgia Entomol. Soc. 11: 105-110.

Shorey, H.H., L.A. Andres and R.L. Hale, Jr. 1962. The biology of Trichoplusia ni (Lepidoptera: Noctuidae). 11. Factors affecting adult fecundity and longevity. Ann. Entomol. Soc. Am. 56: 476-480.

Solbreck, C. 1972. Sexual cycle, and changes in feeding activity and fat body size in relation to migration in Lygaeus equestris (L.) (Het.,Lygaeidae). Ent. Scand. 3: 267-274.

Sparks, A.N., R.D. Jackson, J.E. Carpenter and R.A. Muller. 1986. Insects captured in light traps in the Gulf of Mexico. Ann. Entomol. Soc. Am. 79: 132-139.

Walker, T.J. 1980. Migrating Lepidoptera: Are butterflies better than moths? Fla. Entomol. 63: 79-98.

Weis-Fogh, T. 1952a. Weight economy of flying insects. Trans. Ninth Int. Cong. Entomol. 1: 341-347.

Weis-Fogh, T. 1952b. Fat combustion and metabolic rate of flying locusts (Schistocerca gregaria Forskal). Phil. Trans. Roy. Soc. London. B237: 1-36.

Young, E.C. 1965. Flight muscle polymorphism in British Corixidae: Ecological observations. J. Anim. Ecol. 34: 353-390.

CHAPTER I

CALLING BEHAVIOR OF THE SOYBEAN LOOPER

The following chapter is manuscript 86-17-0139 submitted to Environmental Entomology.

ABSTRACT

Laboratory observations on soybean loopers, (Pseudoplusia includens (Walker)), indicated a calling pattern that was highly variable both within individual females over time and among individuals. However, some trends were apparent. The onset of calling showed temporal advancement over the first three nights after adult emergence; the duration of calling showed a temporal advancement during the first six nights, while the termination of calling receded over the same period then reversed its trend. The majority of females initiated calling three nights following emergence while maximum percent calling was observed around five hours after the onset of scotophase.

Physiological factors such as age and reproductive status, as well as environmental factors, particularly temperature and photoperiod, may affect the diel periodicity of calling (i.e. pheromone release) in many insect species (Sower et al. 1971, Castrovillo and Carde 1979, Bjostad et al. 1980, Alford and Hammond 1982, Mazomenos 1984, Alford and Diehl 1985). Recently the dynamics of calling on reduced time scales have been examined and not surprisingly, calling rhythms can be subdivided into time periods much smaller than a day. Conner et al. (1985) observed Arctiid moths and noted that ovipositors were extruded at frequencies up to 170 extrusions/minute at 25°C. On a somewhat longer time scale, Pseudaletia unipuncta (Haw.), have as many as 16.4 calling bouts per night (Turgeon and McNeil 1982).

Although the soybean looper, Pseudoplusia includens (Walker), can be a pest of soybean and occasionally sweet potato in Louisiana (Hensley et al. 1964), little work has been done on its calling behavior. It is known that virgin female soybean loopers produce sex pheromone between zero and one day post emergence (Shorey et al. 1968), and during late summer, traps baited with virgin females attract the most males between the hours of 12 midnight and 4 A.M. (Mitchell 1973). Lingren et al. (1977) noted that the prereproductive behavior on soybean was quite similar to a closely related species Trichoplusia ni (Hubner). One difference noted was that soybean looper males were more active and circled females before mating. Recently, Alford and Hammond (1982) examined the influence of temperature on the periodicity of soybean looper pheromone release. For every °C decrease in temperature, sex

pheromone release was shifted forward ca. 15 min during the first scotophase, although rhythms returned to normal by the third scotophase. Peak calling at 23°C occurred during the fifth hour of scotophase on day one and during the sixth hour of scotophase on day three This study was undertaken to examine soybean looper calling by first obtaining a general description of calling behavior and lastly, to investigate calling patterns through the quantification of such variables as the number, duration, onset, and termination of calling bouts.

MATERIALS AND METHODS

Pupae were obtained from a second generation laboratory colony, established from field-collected larvae, that had been maintained on a meridic diet (Green et al. 1976). They were sexed, placed in separate 237 ml paper containers and held at 24°C, 65% humidity and a 14:10 LD photoperiod until emergence. Moth behavior was observed using light filtered through a red gelatin filter (Turgeon and McNeil 1982) maintaining the same environmental parameters under which they were reared.

I. CALLING BEHAVIOR:

To observe calling behavior, six newly-emerged females were placed in the experimental chambers (15-cm cube with airflow permitted from the top)(West et al. 1984))(65% RH, 24°C) with a 10% sucrose solution provided as the adult food source. Observations began at the onset of scotophase and continued constantly for at least six hours every night for seven nights. After each cohort, chambers were cleaned and all 'old' females were replaced with newly emerged ones. A total of 48 females was observed to measure variation in calling behavior.

II. QUANTIFICATION OF CALLING PATTERNS:

Twenty newly eclosed females were placed singly in individual experimental chambers. The experiment was replicated over time, three times, for a total of 60 females observed. Observations were initiated at the beginning of scotophase, and continued at one minute intervals until photophase began or calling terminated (whichever occurred last). Termination of calling was determined by the observation of no calling for an hour after the last calling bout. A female was recorded as calling if the pheromone gland was exposed and the wings were held in the characteristic pose described below in part I.

RESULTS AND DISCUSSION

I.CALLING BEHAVIOR:

After the onset of scotophase, the earliest initiation of calling behavior occurred at 65 minutes, while the maximum number of individuals observed calling occurred around 330 minutes. Females usually alighted on a vertical surface and aligned the body axis parallel to the substrate with the head upward. Wings were spread at an approximate 45^{o} angle to the body and wing vibration occurred in 90% of the observations. The abdomen was curved upward away from the vertical surface with the pheromone gland exposed. The females usually did not change location unless disturbed, in which case they flew to another surface and reinitiated calling. Periodically females would stop wing vibrations, withdraw the pheromone gland and remain stationary. Calling by these females would usually be reinitiated within a few minutes.

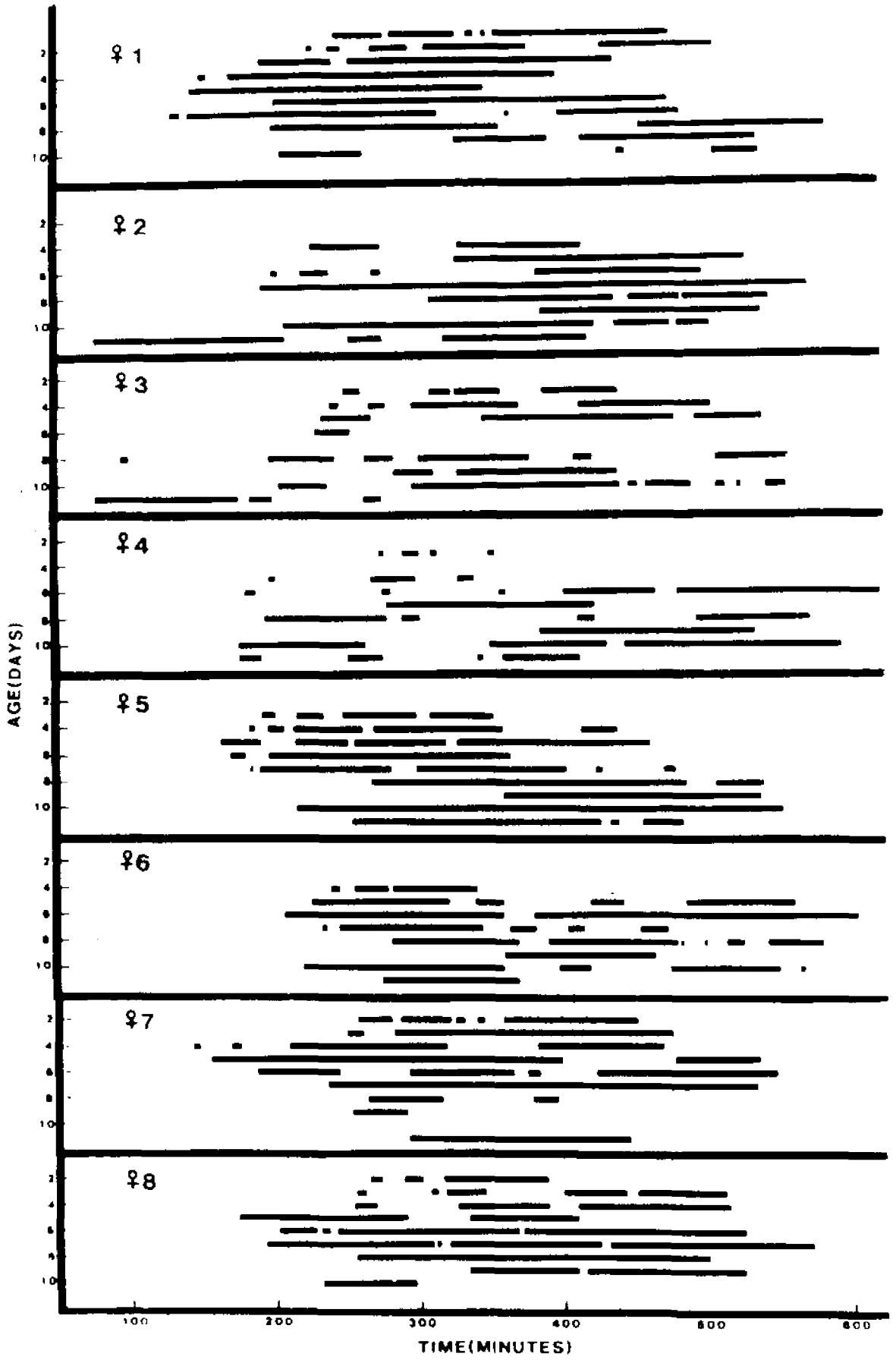
II.QUANTIFICATION OF CALLING PATTERNS:

Calling patterns of females were highly variable both within (over time) and among individuals (Fig. 1), although there were no significant ($F=1.82$, $DF=449$, $P \geq 0.070$)) differences in the number of bouts (Table 1). Twenty-one percent of all nightly calling initiations began with a bout of short duration (< 10 minutes). Subsequent calling bouts in a given night were much longer. Similar behavior has been reported for other noctuids, including P. unipuncta (Haw.)(Turgeon and McNeil 1982) and Hydraecia micacea Esper (West et al. 1984).

The majority of females initiated calling three nights following emergence (Fig. 2). However, the initiation of calling prior to this time (e.g. day one) may be an indication of reproductive maturity at emergence. Sex pheromone production in female P. includens starts at zero to one day post-emergence; mating can occur as early as one day post-emergence and ovarioles contain 50% of the maximum level of chorinated eggs as early as 1.5 days post-emergence (Shorey et al. 1968). Because mating by female soybean loopers has not been reported to occur without pheromone release and the production of viable eggs cannot occur without insemination, it seems reasonable that all these activities would be intimately timed. A relationship between ovarian development and calling has been established for other noctuids (Shorey et al. 1968; Lawrence and Bartell 1972; Swire et al. 1976).

The percentage of virgin females calling at least once during the night reached a maximum of 100% on day five, then declined over

Figure 1. Calling patterns of 8 individual virgin P. includens females on days 1 through 11. Time represents minutes after onset of scotophase.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AGE(DAYS)
100
200
300
400
500
600
TIME(MINUTES)

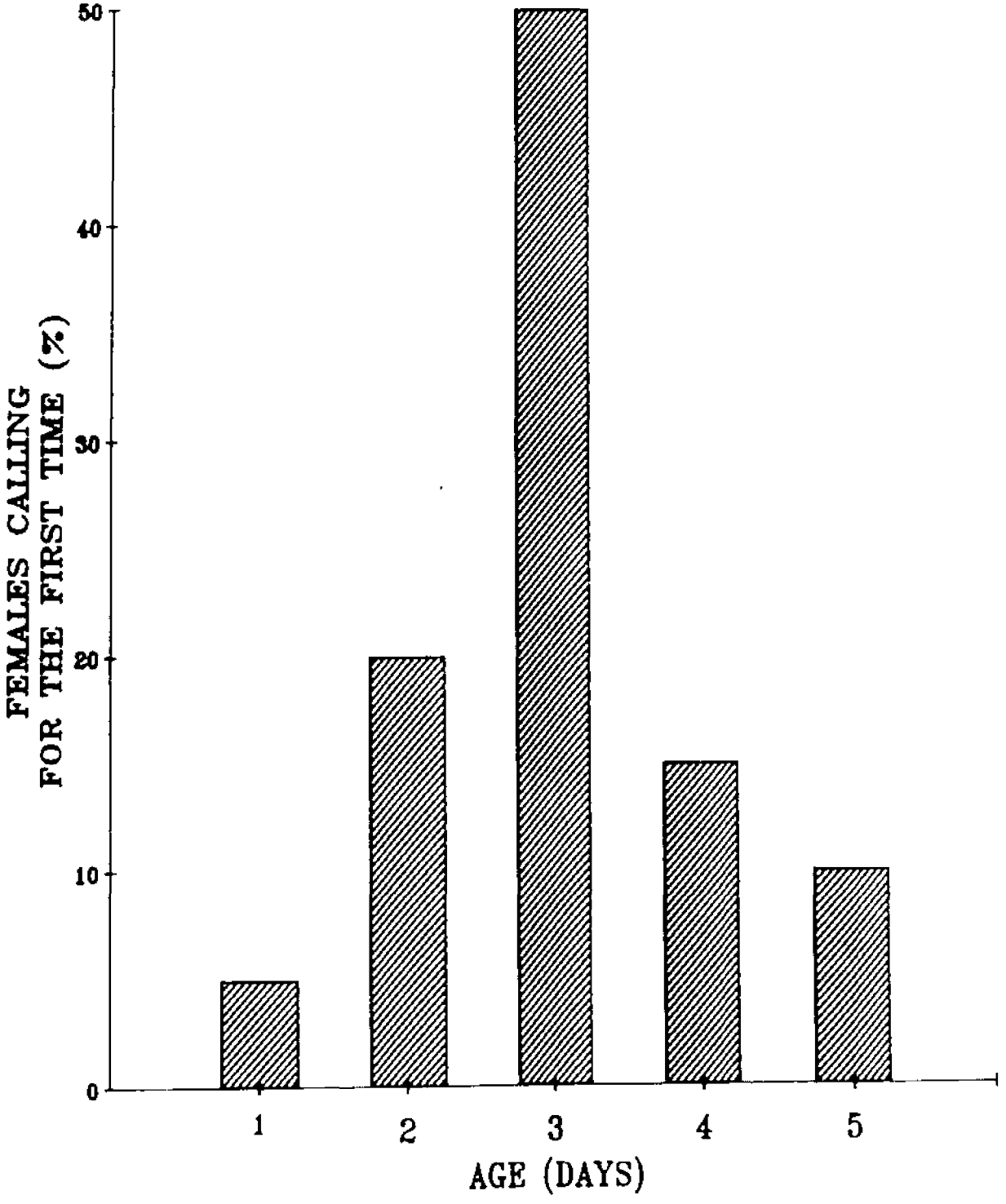
Table 1. Effects of age on calling behavior of virgin P. includens females.

AGE[1] (N)	MEAN NO. BOUTS PER CALLING ♀ $\bar{X}$ ± S.E.[2]	MEAN DURATION (MIN) OF CALLING PER CALLING ♀ $\bar{X}$ ± S.E.	MEAN ONSET TIME (MIN) OF CALLING $\bar{X}$ ± S.E.	MEAN TERMINATION TIME (MIN) OF CALLING $\bar{X}$ ± S.E.
1(60)	3.1 ± 0.3a	99.6 ± 14.3 b	237.0 ± 9.0a	378.9 ± 13.1 d
2(57)	3.2 ± 0.3a	174.5 ± 16.0a	203.3 ± 10.2ab	453.3 ± 14.4 bc
3(57)	2.9 ± 0.2a	197.8 ± 20.8a	183.5 ± 8.7 b	456.0 ± 19.0 bc
4(54)	2.7 ± 0.3a	204.3 ± 20.0a	216.7 ± 13.0ab	468.4 ± 23.9ab
5(51)	3.1 ± 0.4a	222.4 ± 26.7a	222.1 ± 15.0ab	484.2 ± 20.8ab
6(48)	2.1 ± 0.3a	224.8 ± 23.9a	243.3 ± 22.2a	522.6 ± 10.6a
7(51)	2.2 ± 0.3a	179.8 ± 17.0a	247.9 ± 23.2a	482.3 ± 18.5ab
8(42)	2.3 ± 0.4a	186.6 ± 26.7a	222.3 ± 16.7ab	463.3 ± 37.2abc
9(30)	2.2 ± 0.3a	113.3 ± 16.6 b	226.2 ± 21.6ab	400.7 ± 25.6 cd

[1] Calling age (where Day 1 of calling is Age 1), as suggested by Turgeon and McNeil (1982), was used to compare cohorts rather than chronological age.

[2] Mean separation by Duncan's multiple range test (Duncan 1955). For each group, means in a column followed by the same letter are not significantly different ($\alpha = 0.05$).

Figure 2. Post-eclosion ages at which virgin P. includens females initiated calling (N=60).


FEMALES CALLING
FOR THE FIRST TIME (%)
50
40
30
20
10
0
1
2
3
4
5
AGE (DAYS)

the next five days to an average of 85% (Fig. 3). Maximum percent calling at any particular time during the scotophase was 5, 20, 55, 70, 80, 60, 75, 75, 65, 55 and 50 for one to 11 day old females, respectively (Fig. 4).

Maximum percent calling was observed between 270 and 390 minutes after onset of scotophase (Fig. 4) which corresponds to the maximum peak of pheromone release (Alford and Hammond 1982). No calling was observed within the first 60 minutes of scotophase. Females called into photophase on six occasions. This probably was due to the abrupt change from dark to light in the laboratory (i.e., females took a few moments to react to the presence of light). Because light intensity changes gradually at sunrise and sunset in nature, termination of calling in nature probably involves reaction to gradual light intensity changes which was not duplicated in this laboratory study. West et al. (1984) found that H. micacea would call until lights came on in the laboratory, while calling always terminated one hour before sunrise in the field.

The mean duration of calling per virgin female, or the average total time that a female actually called during scotophase, was influenced by age (Table 1). Females on day two through eight called significantly ($F=4.49$, $DF=449$, $P\leq0.0001$) more than on days one and nine. There was a trend toward increased calling time from day two to day six and then decreasing after day six. Similar increasing trends during the first four days have been found for some other noctuids (Swire et al. 1977; Turgeon and McNeil 1982; West et al. 1984). The closely related species, T. ni, spends less time calling as they age (at least to six days), while the average

Figure 3. Percentages of virgin P. includens females calling for different post-emergence ages (N=60).

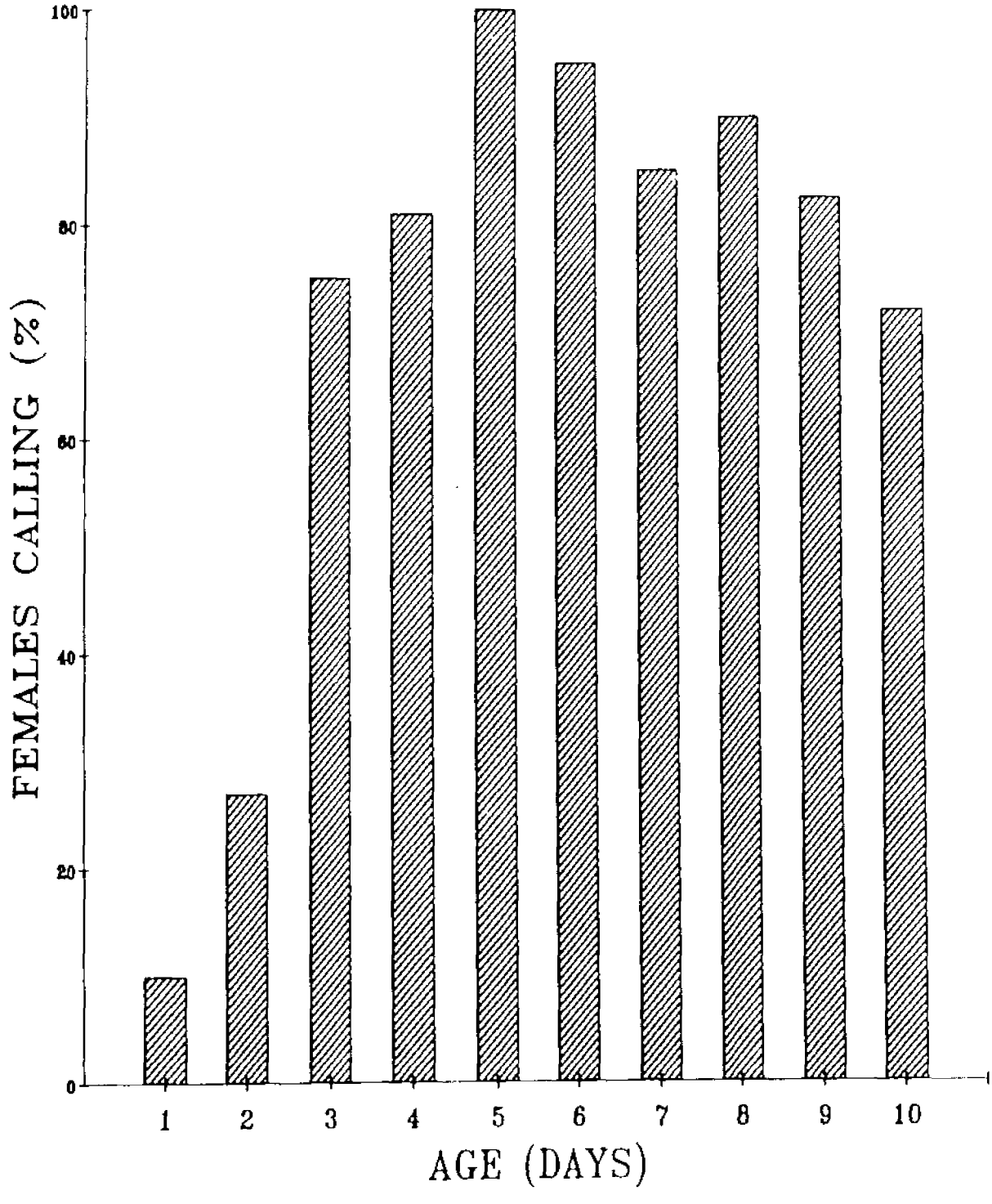
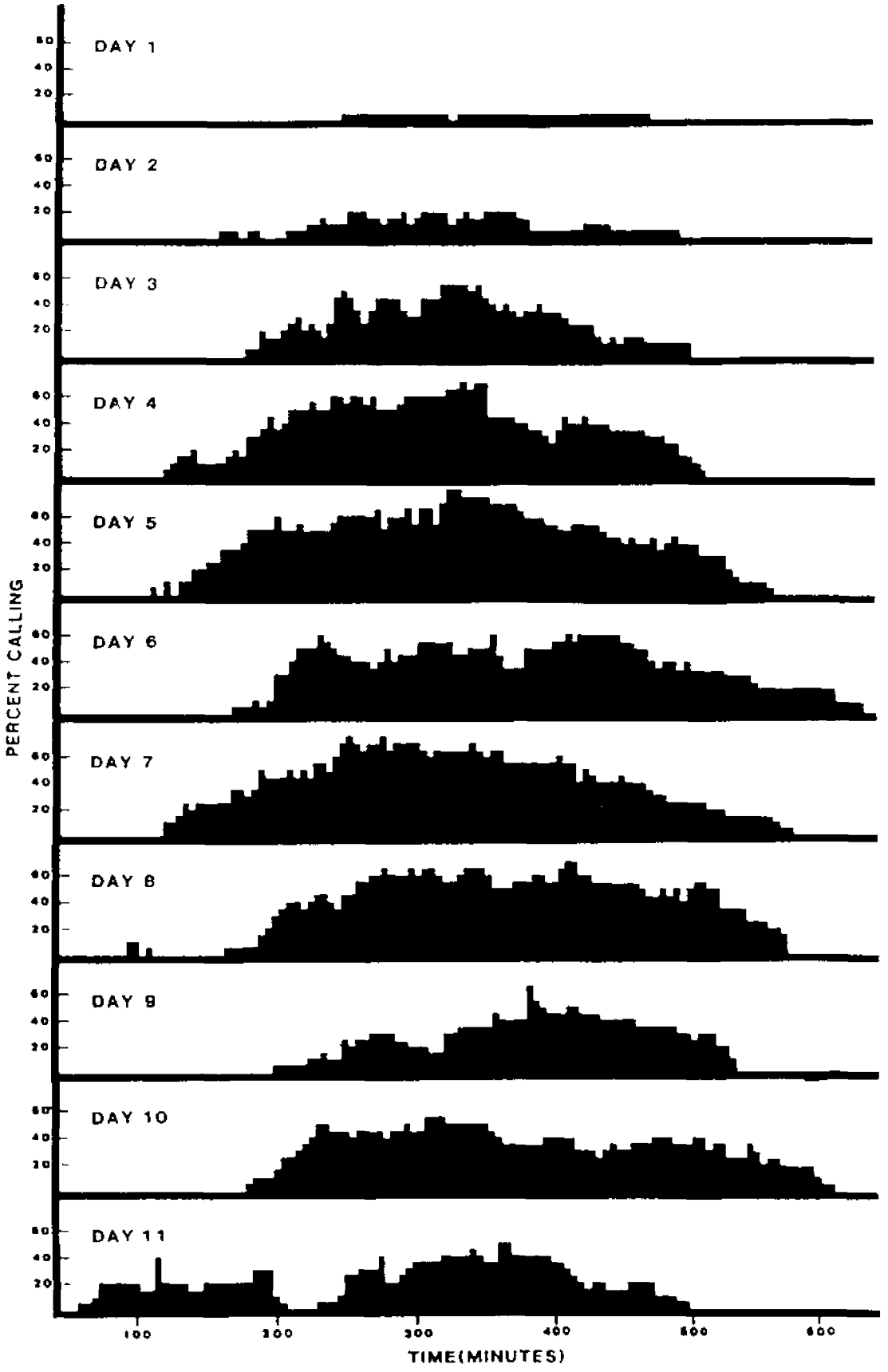

FEMALES CALLING (%)
100
80
60
40
20
0
1
2
3
4
5
6
7
8
9
10
AGE (DAYS)

Figure 4. Percentage of virgin P. includens females calling at 5 min. intervals for days 1 through 11. Scotophase ended at 600 min. (N=60).


DAY 1
DAY 2
DAY 3
DAY 4
DAY 5
DAY 6
DAY 7
DAY 8
DAY 9
DAY 10
DAY 11
PERCENT CALLING
60
40
20
100
200
300
400
500
600
TIME(MINUTES)

rate of pheromone released increases (Bjostad et al. 1980). Senescence may be a cause in the reduction of soybean looper calling and has been suggested as a possible reason for reduced pheromone production and changes in calling patterns in older Epiphyas postvittana (Walk.) females (Lawrence and Bartell 1972).

There were significant ($F=1.84$, $DF=449$, $P\leq0.015$) differences in the mean time of onset of calling. Females calling for their third night called on average 54 minutes earlier than females calling for the first time (Table 1). This trend in temporal advancement has been found in many Lepidoptera including the black cutworm (Agrotis ipsilon) (Swire et al. 1977), the armyworm (P. unipuncta) (Turgeon and McNeil 1982), the sweet potato leaf roller (Brachmia macroscopa) (Hirano and Muramoto 1976) the rice stem borer (Chilo suppressalis) (Kanno 1979) and the currant clearwing moth (Synanthedon tipuliformis (Clerck)) (Buda and Karalius 1985). This temporal advancement of calling may be advantageous to older females in that they are in a position to attract males before younger females begin calling (Swire et al. 1977) or it may be due to age specific changes in pheromone production (Hendrikse 1978). Shorey et al. (1968) demonstrated that pheromone production in T. ni. did not appear to change from day two to day six. In P. includens, it did increase from day zero to day one through three and again on days four through six.

There also were significant ($F=4.51$, $DF=449$, $P\leq0.0001$) changes in the mean time of termination of calling (Table 1). The earliest time of termination was observed on the first day of calling, and it gradually receded to the latest time of termination on night six.

It then advanced slightly until it almost returned to that of day one. Thus, middle aged virgin females do not just expand the time period between onset and termination of calling, they increase the actual time spent calling. This is accomplished by increasing the length of the calling bouts rather than increasing the number of bouts. Early calling older virgins may enhance the probablity of attracting males before young females are available by this onset advancement and increased duration. If these same females are still unmated later in the evening and continue to call after younger ones have terminated calling or mated, then they again enhance their probability of mating by attracting males that have not mated that night (males only mate once per night).

LITERATURE CITED

Alford, A.R. and J.R. Diehl. 1985. The periodicity of female calling in the blueberry spanworm Itame argillacearia Pack. (Lepidoptera:Geometridae). Can. Entomol. 117: 553-556.

Alford, A.R. and A.M. Hammond, Jr. 1982. Temperature modification of female sex pheromone release in Trichoplusia ni (Hubner) and Pseudoplusia includens (Walker)(Lepidoptera: Noctuidae). Environ. Entomol. 11: 889-892.

Bjostad, L.B., L.K. Gaston and H.H. Shorey. 1980. Temporal pattern of sex pheromone release by female Trichoplusia ni. J. Insect Physiol. 26: 493-498.

Buda, V. and V. Karalius. 1985. Calling behavior of females of currant clearwing moth, Synanthedon tipuliformis (Clerck) (Lepidoptera, Sessidae. Z. Ang. Ent. 100: 297-302.

Castrovillo, P.J. and R.T. Carde. 1979. Environmental regulation of female calling and male pheromone response periodicities in the codling moth (Lasperyresia pomonella). J. Insect Physiol. 25: 659-677.

Conner, W.E. , R.P. Webster and H. Itagaki. 1985. Calling behavior in Arctiid moths: The effects of temperature and wind speed on the rhythmic exposure of the sex attractant gland. J. Insect Phsyiol. 31: 815-820.

Duncan, D.B. 1955. Multiple range and multiple F tests. Biometrics 11: 1-42.

Green, G.L., N.C. Leppla and W.A. Dickensen. 1976. Velvetbean caterpillar: a rearing procedure and artifical medium. J. Econ. Entomol. 69: 487-488.

Hendrikse, A. 1978. Factors influencing the courtship behavior of Yponomeuta vigintipunctatus (Retz.)(Lepidoptera: Yponomeutidae). Neth. J. Zool. 28: 139-149.

Hensley, S.D., L.D. Newsom and J. Chapin. 1964. Observation on the looper complex of the noctuid subfamily Plusiinae. J. Econ. Entomol. 57: 1006-1007.

Hirano, C. and H. Muramoto. 1976. Effects of age on mating activity of the potato leaf roller, Brachmia macroscopa (Lepidoptera: Gelechiidae). Appl. Ent. Zool. 11: 154-159.

Kanno, H. 1979. Effects of age on calling behavior of the rice stem borer, Chilo suppressalis (Walker)(Lepidoptera: Pyralidae). Bull. Entomol. Res. 69: 331-335.

Lawrence, L.A. and R.J. Bartell. 1972. The effect of age on calling behavior of virgin females of Epiphyas postvittana (Lepidoptera) and on their pheromone content and ovarian development. Entomol. Exp. Appl. 15: 455-464.

Lingren, P.D., G.L. Greene, D.R. Davis, A.H. Baumhover and T.J. Henneberry. 1977. Nocturnal behavior of four lepidopteran pests that attack tobacco and other crops. Ann. Entomol. Soc. Am. 70: 161-167.

Mazomenos, B.E. 1984. Effect of age and mating on pheromone production in the female olive fruit fly, Dacus oleae (Gmel.). J. Insect Physiol. 30: 765-769.

Mitchell, E.R. 1973. Nocturnal activity of adults of three species of loopers, based on collections in pheromone traps. Environ. Entomol. 2: 1078-1080.

Shorey, H.H., S.U. McFarland and L.K. Gaston. 1968. Sex pheromones of noctuid moths. XIII. Changes in pheromone quantity, as related to reproductive age and mating history, in females of seven species of Noctuidae (Lepidoptera). Ann. Entomol. Soc. Am. 61: 372-376.

Sower, L.R., H.H. Shorey and L.K. Gaston. 1971. Sex pheromones of noctuid moths. XXV. Effects of temperature and pheromone on circadian rhythms of sex pheromone release by females of Trichoplusia ni. Ann. Entomol. Soc. Am. 64: 488-492.

Swire, S.R., R.W. Rings and G.J. Musick. 1976. Reproductive behavior of the black cutworm, Agrotis ipsilon. Ann. Entomol. Soc. Am. 69:546-550.

____. 1977. Age-related calling behavior of the black cutworm, Agrotis ipsilon. Ibid. 70: 919-924.

Turgeon, J. and J. McNeil. 1982. Calling behavior of the armyworm, Pseudaletia unipuncta. Entomol. Exp. Appl. 31: 402-408.

West, R.J., P.E.A. Teal, J.E. Laing and G.M. Grant. 1984. Calling behavior of the potato stem borer, Hydraecia micacea Esper (Lepidopetera: Noctuidae), in the laboratory and the field. Environ. Entomol. 13: 1399-1404.

CHAPTER II

OBSERVATIONS ON THE MATING BEHAVIOR OF *PSEUDOPLUSIA INCLUDENS* (WALKER)(LEPIDOPTERA: NOCTUIDAE)

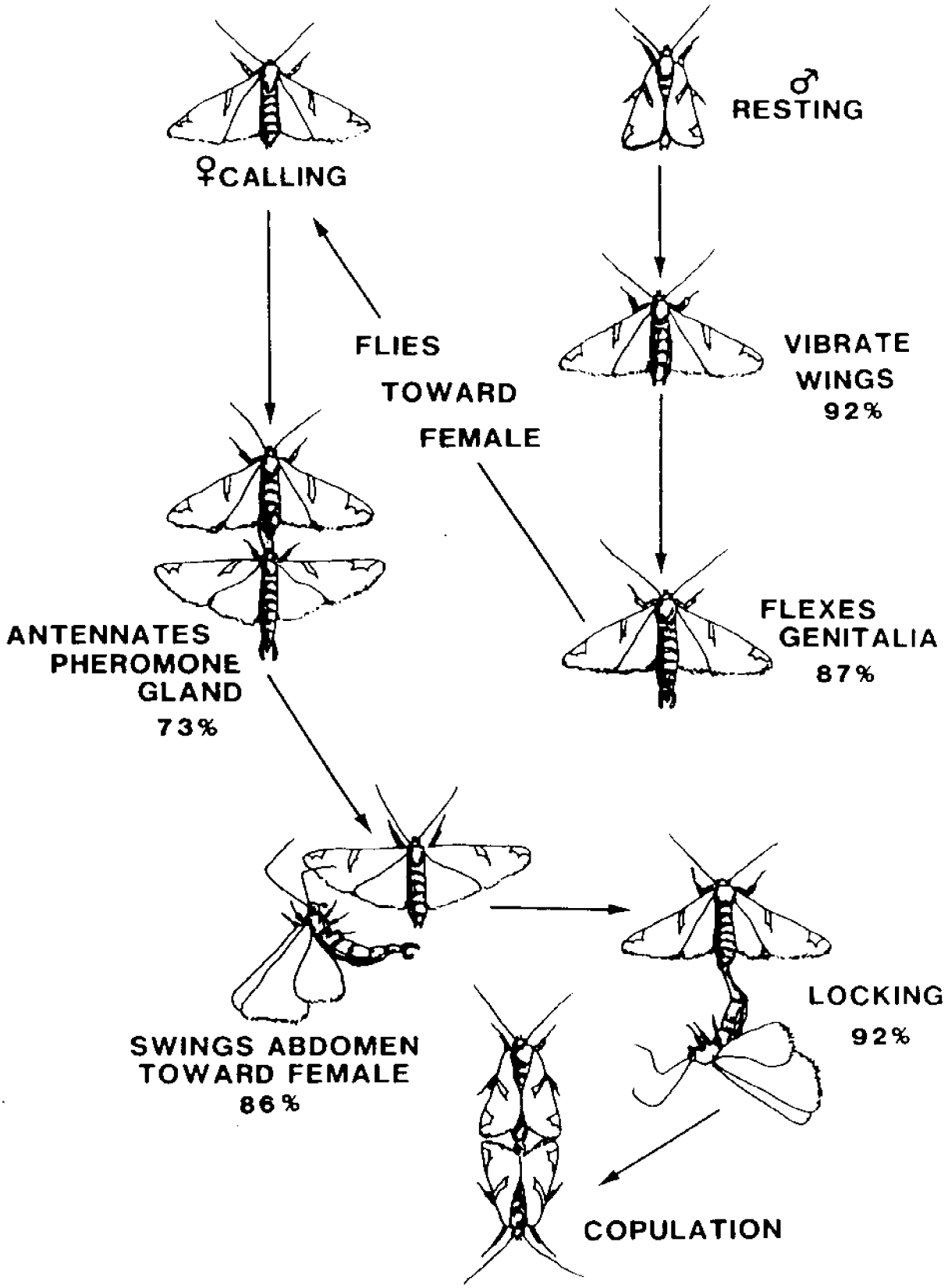
The following chapter is manuscript 86-17-0137 accepted for publication in Florida Entomologist.

In this paper we report results of a soybean looper, (Pseudoplusia includens (Walker)), mating study. To investigate mating behavior, ten 2-day old adults (5♀:5♂) were placed in a container (15 cm^3) and held at 24°C, 65% R.H. and a 14:10 LD photoperiod. Observations were initiated at onset of scotophase and continued for six hours. The experiment was replicated ten times through time. A trial was terminated when 50% of the individuals in a container had died or after 10 days (whichever occurred first). After termination, the bursa copulatrix was dissected in alcohol and spermato- phores counted.

During feeding, oviposition and early calling by females, males either rested on a vertical surface or fed. When males initiated wing and antennal vibration (Fig.1), genitalia were exposed and retracted repeatedly and hair pencils extruded. At this point, the male flew toward a calling female and approached her from the rear. He either landed below her (87%) or hovered above her (13%) and touched his antennae to her exposed pheromone gland. A prospective female either remained in position (62%) or moved away. If she moved away, the male usually followed her (89%) and again attempted mating. If she remained, the male moved up to her side, swung the tip of his abdomen toward her abdomen and continued to expose his genetalia. The male then locked his genitalia with hers and dropped below her. These last two steps occurred almost simultaneously. At this point, wing vibration stopped and abdominal contractions began in both sexes.

Sixty-six out of 71 pairings remained in copula. The remaining five pairings separated in less than two minutes, and it is unlikely

Figure 1. Diagrammatic summary of precopulatory and copulatory behavior of P. includens. Numbers represent percentages of males that showed that behavior and proceeded to next behavior.


♂
RESTING
♀CALLING
FLIES
TOWARD
FEMALE
VIBRATE
WINGS
92%
ANTENNATES
PHEROMONE
GLAND
73%
FLEXES
GENITALIA
87%
SWINGS ABDOMEN
TOWARD FEMALE
86%
LOCKING
92%
COPULATION

that a successful spermatophore transfer occurred. This observation was supported by dissection data. Average duration of copulation was 47.6 ± 2.5 (X ± S.E.) minutes.

Spermatophore counts were highly correlated to observed matings, ($r = 0.8778$) and the mean number of spermatophores for all females was 2.2 ± 0.2 (X ± S.E.) ranging from zero to five. The mean number of observed matings/ female was 2.0 ± 0.2 (X ± S.E.) with a range of one to five. These were similar, but slightly higher, than numbers reported by others for P. includens (Mitchell 1967; Jensen et al. 1974; Leppla et al. 1979).

Pairing relationships were less conclusive. Only two males failed to copulate during the entire observation period. Excluding first matings (i.e. virgin matings), 13 of the 28 subsequent matings occurred with original mates. Thus, it appeared that the sexes were not randomly mating with individuals available to them. Instead, almost half of matings exhibited mate fidelity. Preliminary data (L.J. Mason and D.P. Pashley, unpubl.) suggests that sperm from males mating subsequent to the first male sire few or no progeny. If females derive a source of nutrient from the male spermatophore or accessory gland products, as demonstrated for some other Lepidoptera (Boggs and Gilbert 1979; Boggs 1981; Marshall 1982), multiple mating with the same partner may be advantageous. Because this was a laboratory study, it may not fully describe behavioral relationships in the field due to the complex nature of biological-environmental interactions. Without further laboratory and field studies, the occurrence and function of male mating preferences remain unknown.

One occurrence of aberrant male mating behavior was observed. A six-day-old virgin was seen expelling a spermatophore. When first observed, the spermatophore was halfway out and the remaining half required an additional 45 minutes to be expelled. Electron microscopy of this spermatophore did not reveal any morphological differences between it and spermatophores found in female reproductive tracts. This male never mated successfully, although attempted matings were observed on successive nights. A second spermatophore was noted in the same cage, but it is uncertain which male expelled it.

REFERENCES CITED

Boggs, C.L. 1981. Selection pressures affecting male nutrient investment at mating in Heliconiine butterflies. Evolution. 35: 931-940.

Boggs, C.L. and L.E. Gilbert. 1979. Male contribution to egg production in butterflies: evidence for transfer of nutrients at mating. Sci. 206: 83-84.

Jensen, R.L., L.D. Newsom, and J. Gibbens. 1974. The soybean looper: Effects of adult nutrition on oviposition, mating frequency, and longevity. J. Econ. Entomol. 67: 467-470.

Leppla, N.C., E.W. Hamilton, R.H. Guy, and F.L. Lee. 1979. Circadian rhythms of locomotion in six noctuid species. Ann. Entomol. Soc. Am. 72: 209-215.

Marshall, L.D. 1982. Male nutrient investment in Lepidoptera: What nutrients should males invest? Am. Nat. 120: 273-279.

Mitchell, E.R. 1967. Life history of Pseudoplusia includens (Walker) (Lepidoptera: Noctuidae). J. Georgia Entomol. 2: 53-57.

CHAPTER III

INFLUENCE OF CARBOHYDRATE DEPRIVATION AND TETHERED FLIGHT ON STORED LIPID, FECUNDITY, AND SURVIVORSHIP OF THE SOYBEAN LOOPER (LEPIDOPTERA: NOCTUIDAE)

This chapter is written in the style used by the journal, Physiological Entomology

ABSTRACT

Influence of tethered flight and carbohydrate deprivation on soybean looper (Pseudoplusia includens) whole body lipids, oviposition rate and survivorship was examined using a factorial experimental design. Food had a greater effect than flight on all three variables, although flight did decrease fecundity. Moths that were subjected to only one stress, either two hours of daily flight or provided water as their only food source, were significantly different from both the no-stress group (nonflown and fed) and those that were both starved and flown. The general trend apparent for all parameters was nonflown-fed>flown-fed≈nonflown-starved>flown-starved, except for stored lipids which showed no statistical difference between flown-starved and nonflown-starved moths. Average longevity ranged from nine days for nonflown-fed moths to six days for flown-starved moths. These data demonstrated that the availability of a nectar source may be an important factor in the success of long distance soybean looper dispersal and subsequent reproduction.

INTRODUCTION

For some insects, migration can depress an individuals reproductive potential by increasing the risk of mortality or by diverting energy reserves away from oogenesis (Roff 1977). This would be particularly true when a common energy reserve is used for flight and reproduction. Unless these reserves are regenerated before initiation or continuance of oogenesis, fecundity could be affected. This was particularly true for Drosphilia subobscura where flight significantly decreased fecundity of 10-day-old females for two days following tethered flight (Inglesfield and Begon 1983). Weis-Fogh (1952a) found that in the desert locust, Schistocerca gregaria, overnight feeding did not always replace lipid spent by flying females because ovaries competed for stored lipids.

Energy sources utilized during flight are either fatty acids or carbohydrate (Teo et al. 1987; Van der Horst et al. 1980; Beenakkers 1969). In Lepidoptera, stored lipid is used as the primary flight fuel during long flights (Weis-Fogh 1952b), although carbohydrate may be used initially because it can be mobilized quickly (Van der Horst et al. 1978; Dallmann and Herman 1978). After initial supplies of carbohydrate are depleted, lipid from the fat body and elsewhere is utilized. Lipids must be stored in the egg in sufficient amounts during oogenesis to provide the developing embryo with its major source of energy (Beenakkers et al. 1981). Lipids for oogenesis can be obtained from either fat body stores, directly from ingested food, or synthesis in the ovary (Gilbert 1967). If fat body stores and ingested food are the most important reserves, then oogenesis can be in direct competition with the energetic demands of flight.

The null hypothesis that was tested was that flight and adult diet singly or in concert did not affect either whole body lipid stores, fecundity or survivorship. The alternative hypothesis was that flight and/or adult diet did affect those variables. Through this test we hoped to determine the importance of supplemental carbohydrates (nectar feeding) and migratory activity on the reproductive success of female soybean loopers (Pseudoplusia includens (Walker)), an annual migrant into Louisiana in late spring (Newsom et al. 1980, Johnson and Mason 1985).

MATERIALS AND METHODS

Soybean looper pupae were obtained from a laboratory colony established nine months earlier from field-collected larvae (St. Gabriel, LA). Pupae were sexed, placed in 3.8 L paper containers and held at 28±2°C on a 12:12 LD photoperiod at a RH of > 70%. The day after eclosion, females were chilled and thoracic scales were removed by gentle brushing. A small loop (0.5 cm) of cotton-polyester thread was glued to the thorax with Duro® plastic cement. The procedure required less than a minute. Each female was then were placed with a similar aged male in a 0.5 L paper container. The top was covered with cotton gauze, providing an ovipositional surface, and the bottom of the container was covered with vermiculite. Fed moths were provided a 15% sucrose solution while starved moths were only given water. Males provided for mating were never flown but being in the same container, had access to the same food as females.

Moths were tethered during dusk of the third day after emergence and then again on every subsequent day at dusk until they

expired, escaped or were sacrificed for lipid analysis. Tethering was accomplished by snapping the thread loop into a brass barrel swivel (size 14) that was suspended on a 10 cm length of a cotton-polyester thread attached to a shelf in a walk-in temperature chamber. A split shot sinker (size 4, 3.6 g) was attached to the thread just above the swivel to limit the circumference of flight. Threads were hung 15 cm apart to prevent contact between adjacent moths. To encourage flight a wind current of 10 knots was created with small electric fans. Relative humidity was maintained at > 90% to prevent desiccation and the temperature was 26±2°C. After two hours of flight, moths were returned to their containers to permit egg laying, with egg sheets being replaced every other day. Controls consisted of tethering moths in the usual way, but flight was prevented by allowing tarsal contact with a piece of styrofoam or paper. As with flown individuals, controls were removed from the tether after two hours and their subsequent egg laying was monitored. A total of 811 moths was set up in two reps, but due to deaths, escapes and/or sacrifice only 336 were utilized in lipid determination, 698 for oviposition counts and 383 for surviviorship analysis.

A factorial experimental design was utilized to compare the effects of two stresses, flight and lack of food, on three biological variables - stored lipid, fecundity, and survivorship. Moths were either allowed to fly or not and provided with either water or a 15% sugar solution. The four treatments were (a) Flown-Fed (FF), (b) Nonflown-Fed (NF), (c) Flown-Starved (FS), (d) Nonflown-Starved (NS). Reps were completed approximately three

weeks apart. Ten individuals were sacrificed for lipid analysis per treatment group per run on an alternating day basis to determine lipid accumulation patterns. Sacrificed individuals and eggs were frozen (-15°C) until lipid analysis and egg counts could be made.

Tissues were prepared and lipids analyzed using a modified Bligh and Dyer (1959) technique. This is a one step extraction method, (greater than 98%), for lipids if the monophasic chloroform-methanol subsample size does not produce more than 4 mg lipids/100 ml of the biphasic chloroform-methanol-water mixture. Samples were oven dried (105°C) overnight, weighed, placed in a cup containing 10 ml of a 2% $MgCl_2$ solution, and homogenized with Tekmar® homogenizer. The sample then was transfered to a 100 ml flask where 12.5 ml chloroform and 25 ml methanol were added and blended for two minutes. For 30 mg of dried moths, a 30 ml subsample was removed and then 26.5 ml of a chloroform-methanol-salt water mixture was added to the separatory funnel and shaken vigorously. Fifteen ml of chloroform and 15 ml salt water were then added to the funnel. It was restoppered and shaken again. After 60 minutes the partitioned chloroform layer was drained into tared, dust free aluminum pans. Pans were vacuum dried overnight at 50°C and then re-weighed. Statistical analyses (ANOVA MRT) were conducted using SAS statistical package (SAS Institute 1985).

RESULTS

There were some significant [(day 3, F_{79}=10; P≤0.0001)(day 5, F_{83}=30.9; P≤0.0001)(day 7, F_{65}=11.99; P≤0.0001)(day 9, F_{58}= 21.90; P≤0.0001)(day 11, F_{37}=4.21; P≤0.0230)] differences in the percent of lipid present (Table 1). The treatment that was subjected to no

Table 1. Influence of carbohydrate deprivation and tethered flight on whole body lipids (percent of dry weight) of adult female soybean loopers (P. includens). TRT=Treatment, NF=Nonflown-fed, FF=Flown-fed, NS=Nonflown- starved, FS=Flown-starved.

TRT	$\bar{X}$±S.E. PERCENT WHOLE BODY LIPIDS DAY						
	3	5	7	9	11	13	15
NF	34±1.5A*	33±0.7A	28±1.5A	27±1.8A	20±1.8A	13±1.3	24±1.3
FF	31±2.2AB	28±2.2A	21±1.3 B	25±1.2A	18±2.1AB	NA	NA
NS	22±1.3 C	17±0.7 B	16±1.2 B	15±1.2 B	11±1.9 B	NA	NA
FS	26±1.6 BC	18±1.1 B	19±2.2 B	NA	NA	NA	NA

*Numbers followed by the same letter are not significantly different at the $\alpha = 0.05$ level (Tukeys studentized range (HSD) test) (SAS Institute, 1985). Statistical analyses were performed on arcsin transformed data. Untransformed data are reported here.

stresses, i.e. the nonflown-fed group (NF), was significantly different from the other three treatments on day 7 and always had the highest numerical % lipid (Figure 1a). Individuals that were starved as adults had significantly lower lipids over all days (F_{336}=71.41; P≤0.0001)(Fed 26.5 ±0.4 ($\bar{X}$±S.E.)(Starved 18.6±0.3), while flight as a treatment effect was not significant overall (F_{336}=1.56; P≤0.2130)(Flown 28.9±1.1 ($\bar{X}$±S.E.)(Nonflown 28.1±1.7).

On day 5 and day 7, there were significant [(day 5, F_{498}=5.81; P≤0.0007)(day 7, F_{319}=10.80; P≤0.0001)] differences in the numer of eggs laid between the no stress group (NF) and the other treatments (Table 2). Flight, as an overall treatment effect significantly reduced the total number of eggs females laid (F_{1794}=19.22; P≤0.0001) from 51.6±3.56/48 hrs. ($\bar{X}$±S.E.)for nonflown individuals to 29.9±3.12/48 hrs. for flown individuals.

Fed individuals laid significantly (F_{1794}=61.09 P≤0.0001) more eggs (57.5±3.69/48 hrs.) than starved individuals (18.8±2.27/48 hrs.) and moths with no stresses (NF) often laid considerably more eggs than all other treatments (Figure 1b). Moths subjected to two stresses (FS) laid the least. The ordering of the four treatment groups was NF>(FF≅NS)>FS. Moth oviposition was more affected by starving than flight. This trend was even more apparent if the cumulative egg totals were examined (Figure 1c). Although no significant differences were found on day 3 (F_{697}=1.2; P≥0.3100), nonflown and fed individuals were significantly different there after [(day 5, F_{697}=10.00; P≤0.0001)(day 7, F_{697}=23.68; P≤0.0001)(day 9, F_{697}=30.16; P≤0.0001)(day 11, F_{697}=32.04; P≤0.0001)(day 13, F_{697}=32.61; P≤0.0001)(day 15 F_{697}=32.77;

Figure 1. Influence of food and flight as a function of age (Days) on soybean looper (P. includens); A) Whole body lipids as % of dry weight; B) Average number of eggs oviposited C) Average cumulative egg totals; and D) Survivorship. (- = Nonflown-Fed), (- - = Flown-Fed), (.... = Nonflown -Starved), (--.-- = Flown-Starved).

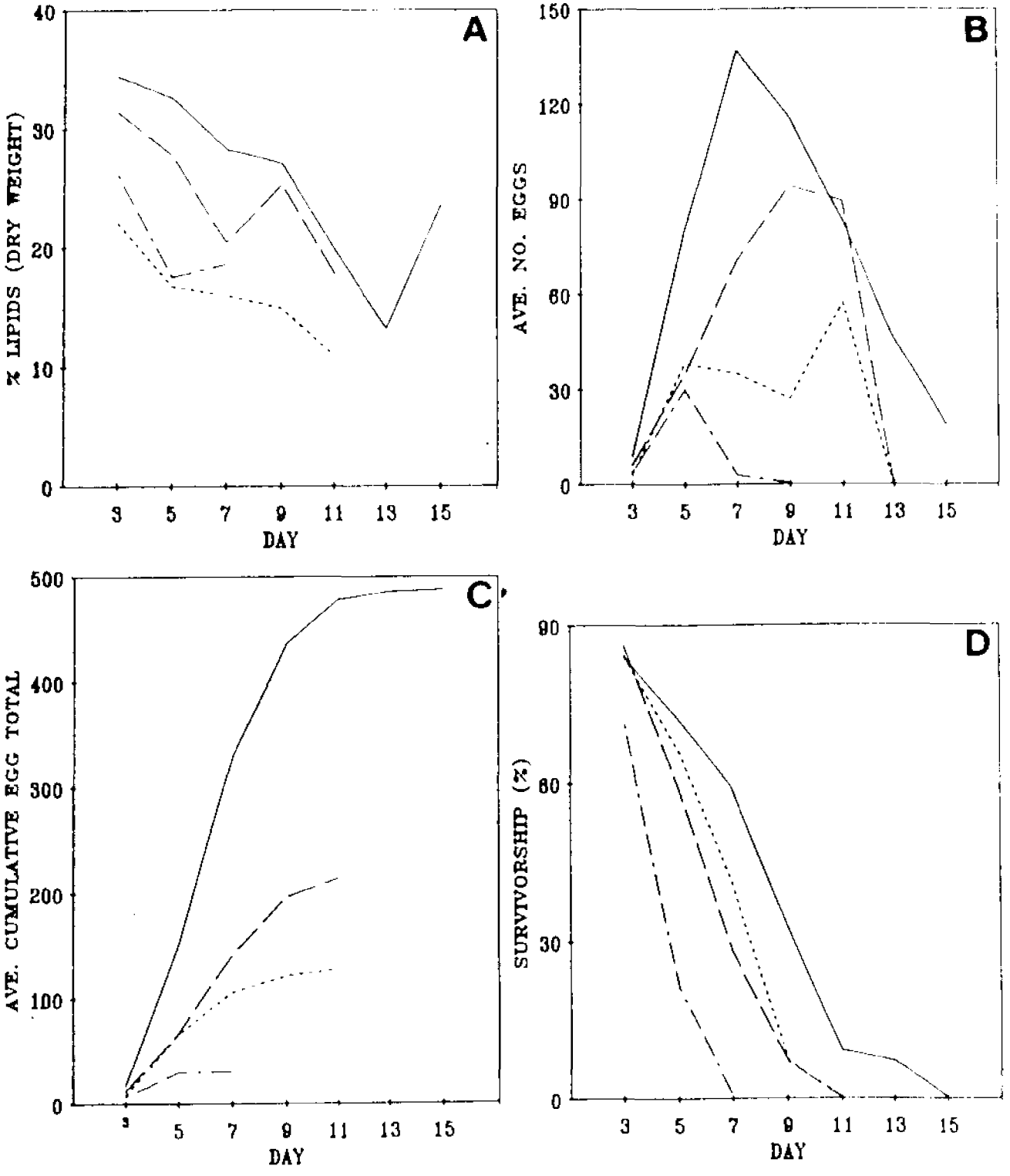

A
% LIPIDS (DRY WEIGHT)
40
30
20
10
0
3 5 7 9 11 13 15
DAY
B
AVE. NO. EGGS
150
120
90
60
30
0
3 5 7 9 11 13 15
DAY
C
AVE. CUMULATIVE EGG TOTAL
500
400
300
200
100
0
3 5 7 9 11 13 15
DAY
D
SURVIVORSHIP (%)
90
60
30
0
3 5 7 9 11 13 15
DAY

Table 2. Influence of carbohydrate deprivation and tethered flight on oviposition of the soybean looper (P. includens). TRT=Treatment, NF=Nonflown-fed, FF=Flown-fed, NS=Nonflown-starved, FS=Flown-starved.

TRT	$\bar{X}$±S.E. EGGS NUMBER DAY						
	3	5	7	9	11	13	15
NF	9±3.6A*	80±12.4A	137±18.1A	115±13.9A	83±16.7A	46±11.6	19±8.2
FF	6±2.4A	35± 7.6 B	71±13.1 B	94±18.1A	89±27.1A	NA	NA
NS	3±1.4A	38± 7.6 B	34± 7.0 B	27± 8.1A	57±32.7	NA	NA
FS	3±1.7A	30±10.1 B	3± 1.0 B	NA	NA	NA	NA

* Numbers followed by the same letter are not significantly different at the $\alpha = 0.05$ level (Tukeys studentized range (HSD) test) (SAS Institute, 1985).

P≤0.0001)) from the other three groups, which together show no statistical differences, except on day 7 when the flown-starved moths were significantly different from flown-fed and nonflown-starved moths. Average age at first oviposition for flown-starved individuals was 5.45±0.379 ($\bar{X}$±S.E.) days. This was lower, but not statistically different (F_{331}=0.89; P≥0.4461) from that of flown-fed, nonflown-fed, and nonflown-starved individuals which were 6.06±0.197 ($\bar{X}$±S.E.), 5.91±0.169, and 5.91±0.157 days old at first oviposition, respectively.

The same relationship apparent in the egg data was present in survivorship data. The nonflown-fed group was significantly different from the flown-fed and nonflown-starved groups, ((NF-FF t_{10}=25.828; P=0.05)(NF-NS t_{10}=28.86; P=0.05). The flown-fed and nonflown-starved groups were not significantly different from each other (t_8=1.079; P=0.05) but they were significantly different from the flown-starved group ((FF-FS t_6=36.239; P=0.05)(NS-FS t_6=40.706; P=0.05)). Examining the point at which 50% or less of the individuals which started the experiment were still alive, yielded the same pattern as with other variables, NF>(FS≡NS)>FS (Figure 1d). The flown-starved moths dropped below 50% survival between days three and five, whereas the flown-fed and nonflown-starved survivorship dropped below 50% survival between days five and seven and nonflown-fed dropped between days seven and nine. Average longevity exhibited similar significant differences (F_{382}=36.49; P≤0.0001). The nonflown-fed group lived an average of nine days while nonflown-starved lived eight, flown-fed seven and flown-starved six.

DISCUSSION

Flight is a very energetically demanding activity for insects. If reproduction and flight use the same energy reserve without replacement, then either reproduction or flight can suffer when reserves are not large enough to supply both. Soybean looper flight behavior is similar to many insects in that the duration of flight decreases with age (Sharp et al. 1976). Although flight propensity was not examined in this study, the interaction of a daily specified flight with a varied food supply was examined.

The general trend found for lipid stores, fecundity and longevity was that nonflown-fed>flown-fed≈nonflown-starved> flown-starved, except for stored lipids which showed no statistical difference between flown-starved and nonflown-starved moths. Starvation seemed to have a greater effect on lipid reserves than flight in P. includens. Although flight did not directly decrease total lipid reserves when compared to nonflown individuals, it did lower egg production. Thus it appears that rather than utilize lipids in oogenesis, lipids are diverted for flight or nonreproductive functions. Even feeding moths did not compensate for the effect of flight on reproduction. It was similarly found that S. gregaria are unable to replace lipid reserves with overnight feeding (Weis-Fogh 1952a). Flight activity of Heliothis virescens severely reduced fecundity and longevity when no supplemental carbohydrate source was provided (Willers 1986).

When flight times are long enough, lipid reduction due to flight has been demonstrated. Spodoptera frugiperda (Van Handel and Nayar 1972 a,b) and Anticarsia gemmetalis (Fescemyer 1986) failed

to show a depletion in whole body lipids or triacylglycerols. It was thought that this was due to a large initial store of lipid, carbohydrate use early in flight, or too short a flight time. S. frugiperda has since been shown to have a 40-58% decrease in whole body lipid with greatly increased flight time (16-30 hrs.), due to new fatty acid not being resynthesized (Van Handel 1974). Observations of Heliothis zea also indicated a decrease (34-85%) in whole body lipid after 23-40 hours of flight (Van Handel 1974).

In summary, food rather than daily flight seemed to have the most pronounced effect on soybean looper lipid reserves, fecundity, and survivorship. Thus, a food source, specifically a supplemental carbohydrate source, can greatly reduce the deletereous influence of flight activity. Availability of a nectar source via weed nectaries resulted in a doubling of ovarian eggs and was thus important in determining the reproductive success of soybean looper in early fall (Collins and Johnson 1985). Flight as an overall effect did not influence lipid reserves, but by competing with oogenesis for lipid, the success of migration could be adversely affected. Nonflown-fed individuals had greater values for all three variables, than those stressed with either a lack of food or flight, while flying and starving individuals simultaneously, greatly reduced their lipid reserves, ability to lay eggs, and survival.

Success of a migration to either establish an overwintering site or merge with a resident population can be partially dependent upon reaching the intended overwintering site with sufficient energy reserves to maintain an overwintering population. Allocation of this limited energy supply to reproductive and nonreproductive

functions must be partitioned accurately. It is not yet determined if the soybean looper is a return migrant. If soybean loopers do have a return migration and a lenghty flight is involved, it appears that intermittent feeding during long flights or soon after flight is terminated is neccessary to prevent a decrease in reproduction.

LITERATURE CITED

Beenakkers, A.M.T. (1969) Carbohydrate and fat as a fuel for insect flight. A comparative study. Journal of Insect Physiology 15,353-361.

Beenakkers, A.M.T., Van der Horst, D.J., and Van Marrewijk, W.J.A.. (1981) Role of lipids in energy metabolism. In Energy Metabolism in Insects. R.G.H. Downer (Ed). Plenum Press N.Y. pp 53-100.

Bligh, E.G. and Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37,911-917.

Collins, F.L. and Johnson, S.J. (1985) Reproductive response of caged adult velvetbean caterpillar and soybean looper to the presence of weeds. Agriculture, Ecosystems and Environment 14,139-149.

Dallmann, S.H. and Herman, W.S. (1978) Hormonal regulation of haemolymph lipid concentration in the monarch butterfly, Danaus plexippus. General Comparative Endocrinology 36,142-150.

Fescemyer, H. W. (1986) The physiological ecology of a migratory Noctuid moth, the velvetbean caterpillar (Anticarsia gemmatalis Hubner). Unpublished Ph.D. Dissertation. Louisiana State University. 139 pp.

Gilbert, G.L. (1967) Changes in lipid content during the reproductive cycle of Leucophaea maderae and effects of the juvenile hormone on lipid metabolism in vitro. Comparative Biochemistry and Physiology 21,237-257.

Inglesfield, C. and Begon, M. (1983) The ontogeny and cost of migration in Drosophilia subobscura Collin. Biological Journal of the Linnean Society 19,9-15.

Johnson, S.J. & Mason, L.J. (1985) The Noctuidae: A case history. In the Movement and Dispersal of Agriculturally Important Biotic Agents. D.R. MacKenzie, C.S. Barfield, G.G.Kennedy, and R.D. Burger. (Eds) Claitor Publ. Baton Rouge, LA. pp 421-433.

Newsom, L.D., Kogan, M., Miner, F.D., Rabb, R.L., Turnipseed, S.G. and Whitcomb, W.H. (1980) General accomplishments toward better pest control in soybeans. In: New technology of pest control. pp. 51-98. C.B. Huffaker (Ed.) John Wiley and Sons, New York, 500 pp.

Roff, D.A. (1977) Dispersal in dipterans: Its cost and consequences. Journal of Animal Ecology 46,443-456.

SAS Institute. (1985) SAS Users Guide. Statistics. SAS Institute Inc. Cary, N.C.

Sharp, J.L., McLaughlin, J.R. and Bennett, D.R. (1976) Flight mill studies with the soybean looper (Lepidoptera: Noctuidae). Journal of the Georgia Entomological Society 11,105-110

Teo, L., Fescemyer, H.W., Woodring, J.P. and Hammond, A.M. (1987) Carbohydrate and fatty acid titers during flight of the migrant noctuid moth, Anticarsia gemmatalis Hubner. Insect Biochemistry (In press)

Van Handel, E. (1974) Lipid utilization during sustained flight of moths. Journal of Insect Physiology 20,2329-2332.

Van der Horst, D.J., Baljet, A.M.C., Beennakkers, A.M.T. and Van Handel, E. (1978) Turnover of locust haemolymph diglycerides during flight and rest. Insect Biochemistry. 8,369-373.

Van der Horst, D.J., Houben, N.M.D. and Beenakkers, A.M.T. (1980) Dynamics of energy substrates in the haemolymph of Locusta migratoria during flight. Journal of Insect Physiology 26,441-448.

Van Handel, E. and Nayar, J.K. (1972a) Turn-over of diglycerides during flight and rest in the moth Spodoptera frugiperda. Insect Biochemistry 2,8-12.

Van Handel, E. and Nayar, J.K. (1972b) Direct use of carbohydrates during sustained flight in the moth Spodoptera frugiperda. Insect Biochemistry 2,203-208.

Weis-Fogh, T. (1952a) Weight economy of flying insects. Transaction of the Ninth International Congress of Entomology 1,341-347.

Weis-Fogh, T. (1952b) Fat combustion and metabolic rate of flying locusts (Schistocerca gregaria Forskal). Philosphical Transactions Royal Society of London. B237,1-36.

Willers, J.L. (1986) Effects of flight and supplemental carbohydrate on fecundity and longevity of Heliothis virescens (Lepidoptera:Noctuidae): An enzymatic and calorimetric approach. Unpublished Ph.D. Dissertation. Mississippi State University. 111 pp.

CHAPTER IV

OOGENESIS-FLIGHT SYNDROME IN *PSEUDOPLUSIA INCLUDENS* (WALKER): EVIDENCE FOR FALL MIGRATION

This chapter is written in the style used by the journal, Ecological Entomology

ABSTRACT. 1. Comparison of daily and seasonal values of total body lipid, proportionate allocation of egg types, and age at first mating were examined in adult soybean loopers, Pseudoplusia includens (Walker).

2. Laboratory moths had a greater percentage of whole body lipids and numbers of chorionated eggs when compared to field-collected moths.

3. Females contained a significantly higher percentage whole body lipid than males.

4. Late season (October and November) females delayed chorionated egg production and had higher non-egg lipids than summer (July, August and September) females.

5. Both sexes contained a greater percentage of whole body lipids late in the fall when compared to early season moths.

6. There was no shift in time of mating or number of matings between seasons.

7. Thus, soybean loopers appear to exhibit a number of the characteristics associated with Johnson's oogenesis-flight syndrome proposed for migratory insects.

Each summer soybean (Glycine max (L.)) growers are faced with a multitude of pests, many of which originate outside the United States. The soybean looper, Pseudoplusia includens (Walker) (Lepidoptera: Noctuidae), an annual migrant into Louisiana in late spring, is only found in the United States in south Florida and south Texas during the winter months (Mitchell et al. 1975, Newsom et al. 1980, Johnson and Mason 1985). A southerly migration possibly occurs in the fall of each year when soybean start to senesce. If there is a southerly fall migration, certain physiological and morphological changes typically associated with migratory species should occur. These changes are part of the oogenesis-flight syndrone (Johnson 1969).

Pre-migrant females prepare for migration by storing large quantites of fat, and delaying egg production and mating (Johnson 1963, Kaster and Showers 1982, Solbreck 1972). Pre-migrant males have no sperm in the seminal vesicles and also show increased fat stores or deposits.

The purpose of this research was to test the hypothesis that adult pre-migratory soybean loopers exhibit the oogenesis-flight syndrome. To test this daily and seasonal values of whole body lipids, proportionate allocation of egg types, and age at first mating of early versus late season female soybean loopers were examined and compared.

MATERIALS AND METHODS

INSECTS: Laboratory and field-collected insects were partitioned according to experimental needs.

LABORATORY: Larvae were fed a pinto bean diet (Green et al. 1976), and adults were provided a 15% sucrose solution. Pupae obtained from a laboratory colony established three generations earlier were utilized in daily lipid analysis and daily ovarian development studies. Pupae were sexed and placed in separate (1 L) emergence containers. Twenty adults were utilized per day in the daily ovarian development study and 12 individuals per day were used in the daily lipid analysis.

FIELD: Field-collected larvae were placed in field cages (1.8 m^3) containing soybeans (Bragg). Pupae collected from field cages, were placed in adult emergence cages (1.5 m x 0.6 m x 0.6 m) in the same soybean field. Newly emerged adults were placed in one of three large field cages (1.8 m^3) that had been cleared of all other insects. Three-day-old adults were removed for subsequent lipid and ovarian analysis. Adults were given access to supplemental 15% sucrose. Twenty field adults were utilized per day in both seasonal lipid and ovarian development studies and 48 individuals per day were used in the mating study. More moths were used in the seasonal lipid study because variation in daily lipid levels was high.

LIPID EXTRACTION: Tissues were prepared and lipids analyzed using a modified Bligh and Dyer (1959) technique. This is a one step extraction method, (greater than 98%), for lipids if the monophasic chloroform-methanol subsample size does not produce more than 4 mg lipids/100 ml of the biphasic chloroform-methanol-water mixture. Samples were oven dried (105°C) overnight, weighed, placed in a cup containing 10 ml of a 2% $MgCl_2$ solution, and homogenized with Tekmar® homogenizer. The sample then was transfered to a 100 ml

flask where 12.5 ml chloroform and 25 ml methanol were added and blended for two minutes. For 30 mg of dried moths, a 30 ml subsample was removed and then 26.5 ml of a chloroform-methanol-salt water mixture was added to the separatory funnel and shaken vigorously. Fifteen ml of chloroform and 15 ml salt water were then added to the funnel, it was restoppered, and shaken again. After 60 minutes the partitioned chloroform layer was drained into tared, dust free aluminum pans. Pans were vacuum dried overnight at 50°C and then re-weighed. Statistical analyses were performed on arcsin transformed data (SAS Institute 1985), but values reported in tables and figures represent untransformed data.

EGG ANALYSIS: Females utilized in this experiment were placed in 80% ethanol until the abdomens were dissected and ovarioles removed. Eggs within four randomly selected ovarioles per female were counted and the number obtained doubled to obtain the number of eggs within all eight ovarioles. Eggs were divided into two catagories. Category 1 - mature eggs with chorion; Category 2 - immature eggs without chorion in the vitellarium and germarium. These two were summed to give the total number of eggs.

EGG LIPID EXTRACTION: Ovarioles were collected from 20 females and used to get an approximation of egg lipids. Eggs were removed from the ovarioles by dissection and placed in 500 ml of a 10% Na_2HPO_4 solution. Eggs were kept suspended by a magnetic stir bar as ten, 10 ml subsamples were removed (with replacement) to determine the average number of eggs in a subsample. Thirty-five subsamples were then removed, drained, weighed, dried overnight at 15°C and the percent lipid was determined using the methods described above. By

subtracting the egg lipid from total lipid the amount of nonegg lipid was determined.

AGE AT FIRST MATING: One hundred ninety-two newly-eclosed adults from emergence cages (1.5 m x 0.6 m x 0.6 m) were placed in one of four screen (1.8 m^3) cages at equal sex ratios and population densities. Starting on day three and continuing daily for eight days, six females and six males were removed from each cage. The bursas were dissected in 80% ethanol and the number of spermatophores were counted. This experiment was replicated during two growing season (1984, 1985) and twice during each year (late July and late October).

RESULTS

The percentage of daily total lipids varied significantly (Male, F_{179}=15.87; P≤0.0001 and Female, F_{179}=23.02; P≤0.0001) as a function of age (Table 1). Female lipids reached a maximum on days 8-11 while male lipids peaked slightly earlier (Days 2-7). Total lipids declined after peaks in both sexes with the exception of a slight increase at the end of the observation period (Day 14 Females; Days 13-14 Males). During the first week there were few differences in total lipid between the sexes while during the second week females generally had more. Overall, females had significantly (F_{345}=99.10 P≤0.0001) more lipid than males (Table 1).

Total lipids also showed significant (Female, F_{99}=37.56; P≤0.0001 and Male, F_{99}=65.47 P≤0.0001) seasonal differences (Table 2). Although total lipids remained the same during the summer months (July, August and September) they increased throughout the fall months (October and November).

Table 1. Daily total percent lipid as a percentage of dry weight for male and female laboratory-reared soybean loopers (*P. includens*) as a function of age.*

	X±S.E. TOTAL% LIPID		X±S.E. % NON-EGG LIPID
DAY	MALE	FEMALE	FEMALE
1	15±0.4 BCDE	13±0.6 E	13±0.6 A
2	18±1.3 ABCD	22±0.6 D	
3	23±2.2 AB	22±0.3 D	17±0.5 A
4	21±1.5 AB	22±1.3 D	
5	23±1.1 A	20±1.5 DE	9±1.6 AB
6	26±11.2 A	22±2.2 D	
7	20±2.5 ABC	23±1.4 CD	9±1.0 AB
8	15±3.0 BCDE	40±0.6 AB	
9	10±1.3 EF	36±1.9 AB	13±3.0 A
10	12±1.6 DEF	44±2.6 A	
11	7±0.5 F	35±2.6 B	5±1.8 B
12	6±0.8 F	20±1.1 DE	
13	15±2.9 BCDE	21±3.5 DE	4±2.3 B
14	12±0.8 CDEF	32±2.9 BC	

* Means followed by same letter are not significantly different (α = 0.05) Tukeys studentized range (HSD) test. Statistical analyses were performed on arcsin transformed data. Untransformed data are reported here.

Table 2. Seasonal differences in the amount of total percent lipid (dry weight) for 3-day-old male and female field-reared soybean loopers (P. includens).*

	$\bar{X}$±S.E. % LIPID		$\bar{X}$±S.E. % NONEGG LIPID
MONTH	FEMALE	MALE	FEMALE
July	7.8±0.3 B	6.5±0.2 C	4.4±0.7 C
August	5.5±0.9 C	5.3±0.4 C	4.0±1.0 C
September	6.4±0.5 BC	5.6±0.3 C	2.8±1.7 C
October	11.5±0.3 A	9.4±0.5 B	8.6±0.7 B
November	13.9±0.3 A	13.5±0.6 A	12.8±0.6A

* Means followed by the same letter are not significantly different (α = 0.05) Tukeys studentized range (HSD) test. Statistical analyses were performed on arcsin transformed data. Untransformed data are reported here.

from days 5-9 while pre-chorionated egg production peaked on day five and remained at that level through day 13 (Table 3). Total number of eggs peaked by day five also, and remained at a high level until day 13. Three-day-old field-collected females showed a significant shift in the type of eggs produced as a function of season. Summer and early fall moths had significantly more chorionated eggs when compared to moths collected in November (F_{99}=30.32 P≤0.0001)(Table 4) despite the fact that pre-chorionated eggs normally are present in numbers ten times the total of chorionated eggs. Thus, fall moths delayed their chorionated eggs production and produced predominatly pre-chorionated eggs. Pre-chorionated eggs can be reabsorbed and thus are available as an energy source is other energy stores become depleted. Total egg production did not change throughout the season.

As with percent lipids, laboratory-reared insects contained significantly (F_{119}=38.4 P≤0.0001) more chorionated eggs when compared to field insects. There were no differences in pre-chorionated and total eggs except during the month of July when field-collected adults had significantly fewer total eggs than laboratory-reared insects.

Chorionated eggs contained 4.07±0.32 ($\bar{X}$±S.E.) percent lipid (dry weight). There were significant differences (F_{82}=6.13 P≤0.0001) in the daily amount of non-egg lipid (Table 1). As the percent total body lipid increased, the proportion of lipid contained in non-egg lipid decreased. The percent total body lipid also varied significantly (F_{99}=782.59, P≤0.0001) with the season (Table 2). Thus, even when the lipids in the chorionated eggs is

Table 3. Type of eggs produced as a function of age in laboratory-reared soybean loopers (P. includens).*

DAY	CHORIONATED $\bar{X}$±S.E.	PRE-CHORIONATED $\bar{X}$±S.E.	TOTAL $\bar{X}$±S.E.
1	0±0 D	1109±19 C	1109±19 D
3	166±6 C	1403±20 B	1569±22 C
5	373±15 A	1636±28 A	2010±35 A
7	371±15 A	1643±30 A	2014±30 A
9	387±14 A	1642±43 A	2029±41 A
11	239±12 B	1668±29 A	1907±29 AB
13	166±3 C	1660±21 A	1826±21 B

* Means followed by the same letter are not significantly different

(

Table 4. Type of eggs produced as a function of month collected for 3-day-old field collected soybean loopers (P. includens).*

MONTH	CHORIONATED $\bar{X}$±S.E.	PRE-CHORIONATED $\bar{X}$±S.E.	TOTAL $\bar{X}$±S.E.
July	130±4 A	1336±22 B	1467±23 A
August	126±3 A	1350±23 B	1476±23 A
September	128±4 A	1356±25 B	1484±25 A
October	135±5 A	1354±29 B	1490±28 A
November	80±4 B	1476±24 A	1555±24 A

* Means followed by the same letter are not significantly different ($\alpha = 0.05$) Tukeys studentized range (HSD) test.

taken into account, November females still have significantly more stored lipid than their summer or early fall counterparts.

There were no differences in the age at first mating or number of matings between 1984 and 1985 so yearly data were pooled. Tests for differences between two independent regressions on pooled data indicated that there was no difference in age at first mating (t_{12}=0.1015; P = 0.05) or number of matings (t_{12}=0.2806; P = 0.05) between July moths and October moths (Table 5). Thus, although late season females delay egg maturation, they do not appear to be delaying mating.

DISCUSSION

Examination of migratory insects indicates that most migration occurs in young female adults just prior to oogenesis and mating, while male migration occurs with no sperm in the seminal vessicles (Johnson 1969). Both sexes tend to accummulate fat. Thus, migratory potential is maximized by reducing reproductive system weight and maximizing fuel supplies and flight systems (wing span, wing muscles). The patterns of lipid reserves and allocation of egg types indicate that soybean loopers appear to comform to the oogenesis-flight syndrome.

Analysis of daily total lipids revealed a pattern in which females were slower than males to reach a peak in total percent body lipids, and overall had higher percent lipids than males. This is unlike most lepidoptera previously examined. Gilbert and Schneiderman (1961) studied total lipid sexual dimorphism in 24 species of Lepidoptera and found that male abdomens may have up to nine times more ether-extractable lipid per gram wet weight than

Table 5. Percent females mated and number of spermatophores per day for early (late July) and late season (late October) soybean loopers (*P. includens*).

	% MATED		$\bar{X}$±S.E. SPERMATOPHORES/FEMALE	
DAY	JULY	OCTOBER	JULY	OCTOBER
3	54	50	0.54±0.10	0.50±0.10
4	75	79	0.88±0.13	0.83±0.10
5	75	83	1.04±0.15	1.21±0.15
6	83	83	1.46±0.20	1.50±0.19
7	83	83	1.67±0.21	1.46±0.19
8	92	88	1.75±0.24	1.96±0.25
9	92	96	2.04±0.24	1.88±0.17
10	92	92	2.08±0.23	1.96±0.23

females. Soybean looper percent total lipids seem to follow trends typically found in non-lepidopterans. In both Periplaneta americana (Munson and Gottlieb 1953) and Acheta domestica (Lipsitz and McFarlane 1970), females, rather than males, generally possess a higher percent lipid.

Seasonally, both sexes increased total lipid levels during the fall. This increase in lipid reserves may be an indicative of preparation for a fall diapause and migration. Since flight is one of the most energetically demanding activites and the primary flight fuel in Lepidopterans is lipid, large stores are expected in insects that are entering diapause and or preparing for migration. Examples of such lipid accumulation include Lygaeus equestris (Solbreck 1972), Nezara viridula (Kiritani 1963), Agrostis infusa (Common 1954) and Danaus plexippus (Beall 1948).

Laboratory-reared insects had considerably more lipids and eggs than similarly aged field-reared insects. This is an example of how colonization can cause dramatic phenotypic changes in laboratory insects (For a review, see Mason et al. 1987). Laboratory-reared insects are provided with a balanced artificial diet and maintained in a less stressful environment while field-reared insects are subjected to a variety of environmental stresses in addition to predators and parasites. Genetic effects also can influence laboratory selection. Although insects used in this study were only three generations removed from the field, bottlenecks and selection can occur rapidly in the laboratory and thus field-reared insects should be used when possible.

Johnson (1969) suggested that one of the ways that pre-migrant females may prepare for migration is by delaying egg production. It appears that if soybean loopers are preparing for a fall migration, it does not stop egg production completely, rather it just slows further egg maturation after vitellogenesis and some oogenesis has occurred. Oncopeltus fasciatus delays reproduction when reared under fall-like conditions (Rankin and Rankin 1979), and it is thought that juvenile hormone is the factor which serves to coordinate long distance flight initiation and ovarian development (Rankin 1980). Other insects that appear to use this strategy include Hippodamia convergens and possibly Danaus plexippus and Leptinotarsa decemlineata (Rankin and Rankin 1979). Kaster and Showers (1982) speculated that adult Agrotis ipsilon enter a reproductive diapause prior to their autumn migration.

The combination of seasonal changes in lipid content and shifts in type of egg production in spite of the lack of delayed mating tend to suggest that female soybean loopers exhibit a majority of the changes generally associated with the oogenesis-flight syndrome.

Mating status is normally not as regulated as oogenesis and lipid accumulation. Mating can occur either at the source, prior to migration (Lewis 1965), during migration (Hodek 1967) or at the overwintering site (Kiritani 1963). Therefore, male migration habits are more variable than female. If supplemental matings are necessary, as is probably the case for L. equestris (Solbreck 1972), then males may migrate with females. Since male soybean loopers did increase their lipid reserves during the months of October and November, they may be accompaning females. A possible explaination

for male soybean loopers to accompany females is that first mating in Lepidoptera are often infertile. (Taylor 1967, Pliske 1973), occurring before sperm are in the spermatophore. If a high incidence of this is occurring in field populations, secondary matings may be important to a high mating success. Males are probably not delaying spermatogenesis since females were not changing either the time of mating or the number of matings.

In conclusion, there appears to be a physiological basis for there being a fall migration of soybean loopers to southerly overwintering sites. Examination of moths throughout migratory routes and at overwinterings sites is necessary to further elucidate the details of this complex life history strategy.

LITERATURE CITED

Beall, G. (1948) The fat content of a butterfly, Danaus plexippus Linn., as affected by migration. Ecology. 29,80-94.

Bligh, E.G. & Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology. 37,911-917.

Common, I.F.B. (1954) A study of the ecology of the adult Bogong Moth, Agrotis infusa (Boisd.) (Lepidoptera: Noctuidae), with special reference to its behaviour during migration and aestivation. Australian Journal of Zoology. 2,223-263.

Gilbert, L.I. & Schneiderman, H.A. (1961) The content of juvenile hormone and lipid in Lepidoptera: Sexual differences and developmental changes. General and Comparative Endocrinology. 1,453-472.

Green, G.L., Leppla, N.C. & Dickensen, W.A. 1976. Velvetbean caterpillar: a rearing procedure and artifical medium. J. Econ. Entomol. 69: 487-488.

Hodek, I. (1967) Bionomics and ecology of predaceous Coccinellidae. Annual Review of Entomology 12,79-104.

Johnson, C.G. (1963) Physiological factors in insect migration by flight. Nature. 198,423-427.

Johnson, C.G. (1969) Migration and dispersal of insects by flight. Methuen and Co., Ltd. London 763 pp.

Johnson, S.J. & Mason, L.J. (1985) The Noctuidae: A case history. In the Movement and Dispersal of Agriculturally Important Biotic Agents. D.R. MacKenzie, C.S. Barfield, G.G.Kennedy, and R.D. Burger. (Eds) Claitor Publ. Baton Rouge, LA. pp. 421-433.

Kaster, L. Von & Showers, W.B. (1982) Evidence of spring immigration and autumn reproductive diapause of the adult black cutworm in Iowa. Environmental Entomology. 11,306-312.

Kiritani, K. (1963) The change in reproductive system of the southern green stick bug, Nezara viridula, and its application to forecasting of seasonal history. Japanese Journal of Applied Entomology and Zoology 7,327-337.

Lewis, T. (1965) The species, aerial density and sexual maturity of Thysanoptera caught in mass flights. Annals of Applied Biology. 55,219-225.

Lipsitz, E.Y. & McFarlane, J.E. (1970) Total lipid and phosphoplipid during the life cycle of the house cricket, Acheta domesticus (L.) Comparative Biochemistry and Physiology. 34,699-705.

Mason, L.J., Pashley, D.P. & Johnson, S.J. (1987) The laboratory as an altered habitat: Phenotypic and genetic consequences of colonization. Florida Entomologist. In Press.

Mitchell, E.R., Chalfant, R.B., Greene, G.L. & Creighton, C.S. (1975) Soybean looper populations in Florida, Georgia, and South Carolina, as determined with pheromone-baited BL traps. Journal of Economic Entomology. 68,747-750.

Munson, S.C. & Gottlieb, M.J. (1953) The differences between male and female American roaches in total lipid content and in susceptibility to DDT. Journal of Economic Entomology. 46,748-802.

Newsom, L.D., Kogan, M., Miner, F.D., Rabb, R.L., Turnipseed, S.G. & Whitcomb, W.H.. 1980. General accomplishments toward better pest control in soybeans. Pp. 52-98. In C.B. Huffaker (Ed.) New technology of pest control. John Wiley and Sons, New York, 500 pp.

Pliske, J.E. 1973. Factors determining mating frequencies in some new world butterflies and skippers. Annals of the Entomological Society of America. 67,871-876.

Rankin, M.A. (1980) Effects of precocene I and II on flight behavior in Oncopeltus fasciatus, the migratory milkweed bug. Journal of Insect Physiology. 26,67-73.

Rankin, M.A. & Rankin, S.M. (1979) Physioloical aspects of insect migratory behavior. Pp. 35-63. In R.L.Rabb and G.G. Kennedy (eds.), Movement of highly mobile insects: concepts and methodology in research. University Graphics, North Carolina State University, Raleigh.

SAS Institute. (1985) SAS Users Guide. Statistics. SAS Institute Inc. Cary, N.C.

Solbreck, C. (1972) Sexual cycle, and changes in feeding activity and fat body size in relation to migration in Lygaeus equestris (L.) (Het.,Lygaeidae). Entomology Scandinavia. 3,267-274.

Taylor, O.R., Jr. 1967. Relationship of multiple mating to fertility in Atteva punctella (Lepidoptera: Yponomeutidae). Annals of the Entomological Society of America. 60,583-590.

SUMMARY

An investigation of the oogenesis-flight syndrome of Pseudoplusia includens (Walker) was conducted with respect to age and seasonal influences. Emphasis was placed on (1) normal calling and mating behaviors (2) influence of tethered flight and carbohydrate deprivation on lipid reserves, oviposition and survivorship and (3) the seasonal variation of lipid reserves, proportionate allocation of egg types and mating status.

Pre-migratory insects often delay mating, especially when males will accompany females on their migratory flights. Although this study did not examine seasonal variation in calling behavior some studies indicate that calling and mating is temporally delayed in pre-migrant females. It was determined that there was no mating delay in late season moths which indicated that both sexes remained sexually active late in the season and could indicate that males are not required for mating at overwintering sites. However, lipid reserves increased in late season males and thus they appear physiologically to be pre-migrants. Future studies should examine reproduction in late season and overwintering populations to determine the role males may play in the overwintering ecology of this species.

It also was determined that there is an energetic cost of flight, and, thus, to reproduction, and that a food source is very important in reducing the effects of flight on reproduction, lipid reserves and survivorship. Soybean loopers show a delay in egg chorionation and thus, they delay egg maturation and use lipid

stores directly for flight and other non-reproductive functions. In this way, females that arrive at overwintering sites have possibly mated or are ready to mate, and already have immmature eggs ready for chorionation. The size of the ovarioles with only immature eggs was considerably smaller than those with a full compliment of mature chorionated eggs. Again, if future studies examine the reproductive strategies used during migratory flights and at overwintering sites, the relative importance of egg maturation to mating could be more fully understood.

The importance of a nectar source to egg production has already been determined for a fall field population of soybean loopers. Depending on the migration route taken, nectar sources may or may not be available. Those that might cross the Gulf of Mexico would probably have less opportunity for nectar replenishment during flight that those following mainland routes.

One important observation from this study is the reinforcement of a recently established idea, that being, that laboratory-reared insects are not always excellent models for their field progenitors. When possible, laboratory insects should be used within a few generations after being brought into the laboratory. If this is not possible, then testing and close monitoring for behavioral and physiological changes is highly recommended.

This investigation has shown that there is a cost to flight (at least daily flight) on reproduction, that late season moths delay reproduction but not mating, that lipid reserves increase through-out the season and that females possessed more total lipids than males. These data strongly suggest the existance of an

oogenesis-flight syndrome in female soybean loopers. Although males show seasonal shifts in the level of their lipid reserves, no shift in mating was observed and the cost of flight on sperm storage was not measured. Thus, the existence of a "spermatogenesis-flight" syndrome is unknown. Further seasonal studies on male reproductive parameters as well as an examination of overwintering populatons might result in a clearer understanding of a "spermatogenesis-flight" syndrome and other important questions.

VITA

Linda Jean Mason was born in Schenectady, New York on February 21, 1956. She is the daughter of Gerald Keith and Jean Marie Mason and has one brother, LCDR Gerald Alan Mason. She attended elementary school in several states within the United States, but spent the entire four years of high school at Nova High School in Davie, Florida. After graduation in May 1974, she attended the University of Florida, Gainesville, Florida and received the Bachelor of Science Degree in Forest Resources and Conservation in June, 1979. She continued her education at Auburn University in Auburn, Alabama, where she received a Master of Science Degree in Entomology in June, 1983. Currently she is a candidate for the Doctor of Philosophy Degree in Entomology at Louisiana State University in Baton Rouge, Louisiana. In February 1987, she started a postdoctoral research position at the University of Florida Tropical Research and Education Center in Homestead, Florida.

DOCTORAL EXAMINATION AND DISSERTATION REPORT

Candidate: LINDA JEAN MASON

Major Field: ENTOMOLOGY

Title of Dissertation: A SEASONAL AND ONTOGENETIC EXAMINATION OF THE REPRODUCTIVE BIOLOGY OF PSEUDOPLUSIA INCLUDENS (WALKER)

Approved:

Major Professor and Chairman

Dean of the Graduate School

EXAMINING COMMITTEE:

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